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THE ORIGIN OF SELF-COMPATIBILITY IN RYE

Data collected by *N. HERIBERT NILSSON*

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I. INTRODUCTION

IN the years 1912—1927 a vast material on self-fertility in rye was collected by HERIBERT NILSSON. Part of it was published in 1916 and demonstrated clearly the presence of heritable degrees of ability to set self seed both within and between the different rye varieties investigated. The predominant majority of the plants in the outbred populations being highly self-sterile, it was an observation of especial interest that highly self-fertile plants were obtained upon selfing from parents with poor self-set and that these offspring plants bred true to self-fertility. Not until 1953 was the origin of such deviating plants made subject to a further discussion by HERIBERT NILSSON, on a limited material, the detailed discussion being postponed to an intended comprehensive report on the biology of self seed-setting in rye. On his death in the summer of 1955 the unpublished data were preliminarily tabulated, and it was considered desirable that the results of this investigation should be made available. Data published by LUNDQVIST (1958) on self seed set in the variety Steel-rye, which was not investigated by HERIBERT NILSSON, have accentuated this need.

It would have been preferable to present the posthumous material in a way that might correspond to the working hypotheses of the collector and to postpone to a separate paper conclusions that might be drawn from these data by an outsider. HERIBERT NILSSON's view on the subject under investigation, however, was formulated in a rather general way (1953), and no complementary impressions from personal discussions were available to the present author. In order to arrive at the necessary condensation of the tabulated material the present author has applied from the beginning his own hypotheses to the data. Therefore, it should

be emphasized that he alone is responsible for the conclusions drawn in this paper.

II. THEORETICAL BACKGROUND

Self- and cross-incompatibilities in rye result from a positive inhibitory reaction between like specificities in pollen and pistil tissue (for details, see LUNDQVIST, 1954, 1956, 1957). The incompatibility specificities are elaborated in a complementary way by two multiallelic loci, named S and Z, which are inherited independently of each other. There are formed as many different S—Z products as there are different S—Z pair-combinations possible in the tissue in question. From, say, the genotype $S_{1\text{-}2}Z_{3\text{-}4}$, four different S—Z products are formed (S_1Z_3, S_1Z_4, S_2Z_3, S_2Z_4), each of them, independently of the other products, being able to bring about an incompatibility reaction between the pistil and an appropriate pollen grain.

It is not known how the complementary products formed in pistil and in pollen and reacting with each other are related to one another. Undoubtedly, the pollen specificities are determined autonomously by the S and Z factors carried in the pollen grain itself. This gametophytic determination of the pollen specificities means that a haploid pollen grain, which carries one S and one Z factor, will have a single S—Z product. This pollen will effect fertilization even when it has either the S or Z factor, but not both, in common with the pistil.

Self-compatible plants may be obtained at a low frequency after selfing of a self-incompatible parent. Their origin is obscure when a test cross to the parent plant is not possible. New specific combinations of modifier factors acting at the level of the offspring pistil could conceivably effect an impairment of the incompatibility reaction. It should be observed that such modifier factors must have their action at the pistil level, as otherwise all pollen carrying this combination of factors would function on the self pistil and render also the parent plant self-compatible.

Extensive data from outbred and inbred populations of diploid Steel-rye, however, did not speak in favour of modifier segregation affecting the incompatibility reaction. They led to the conclusion that highly self-fertile plants in the self offspring from parents with low self-set were due to factors that were not present in the parent and involved the incompatibility loci S and Z (LUNDQVIST, 1958). The rate of self-compatible pollen grains from self-incompatible plants in outbred populations was computed to lie somewhere between 10^{-5} and 10^{-7}.

In one case such self-compatible offspring could be backcrossed to the parent plant and the self-compatibility analysed in some detail (LUNDQVIST, 1958). A new factor was recovered at the Z locus which, judging from its functional pattern, still formed the original S—Z products in the pistil but did not form them in the pollen. A plant carrying such a new factor will become self-compatible and compatible as pollinator on the self-incompatible parent but may be incompatible to all pollen from this plant.

In the absence of evidence to the contrary this new factor was judged to be due to mutation. In any case such a factor cannot be an ordinary constituent of the parental genotype, as this plant, also, would otherwise become self-compatible. In view of the unpublished work of DENWARD (Swedish Seed Association, Svalöf), on self-incompatibility in *Trifolium pratense*, regard must be paid to the possibility that such factors may also be due to recombination within the incompatibility gene.

III. MATERIALS AND METHODS

Not all the collected data lent themselves to an analysis of self seed set. In some cases the origin of a material could not be sufficiently traced; and part of the data seemed too fragmentary to deserve consideration. About three-fourths of the material will, however, be presented here.

Petkus rye with outbred and inbred populations from several selections constitutes the main material. Two other varieties, Storm-rye II and Star-rye, are derived from Petkus rye. The Brattingsborg rye is a Danish indigenous rye. All materials were grown in the experimental fields of Weibullsholm at Landskrona, 30 km. north-west of Lund, and the data were collected in the years 1919—1927.

All isolations were performed in double pergamine bags (HERIBERT NILSSON, 1953). Judging from the profuse tillering the plants must have been sparsely spaced, probably according to previously described methods (HERIBERT NILSSON, 1916, p. 5).

In the present tabulations different varieties and generations of inbreeding are kept separate. In view of the sometimes low numbers of individuals observed, no general attempt will be made here to study separately the data from different years and selections within a variety. Beyond I_s the representativeness of the offspring material is so narrowed as to require its omission even in the Petkus rye. Thus, an offspring generation based on less than 15 parent plants from the original outbred

TABLE 1. *Self seed set in outbred populations of diploid rye varieties.*

Variety	Plant self seed set in per cent							No. of plants	Per cent plants	
	0	0	5	10	20	50	100		below 5 % self-set	out of these with no self-set
Petkus rye	138	553	23	12	1	1		728	94.9	20.0
Storm-rye II	11	46	1	1				59	96.6	19.3
Star-rye	3	34	2	1				40	92.5	8.1
Brattingsborg rye	14	44	3	—	1			61	95.1	24.1
Brattingsborg × Schlaraffen rye	5	19	2	1				27	88.9	20.8

population will not be included here. The statistical calculations are in accordance with BONNIER and TEDIN (1940 and 1957). The adjusted χ^2_c is used in cases of two samples and one degree of freedom, but in doubtful cases the exact probabilities are calculated according to BONNIER and TEDIN (1940, pp. 226—227).

IV. PATTERNS OF SELF-FERTILITY IN OUTBRED AND INBRED POPULATIONS

The patterns of seed set upon selfing in outbred populations — which will be named I_0 according to the terminology of HERIBERT NILSSON (1953) — are given in Table 1 for the four investigated varieties and a hybrid population. No values from open seed set are available as a control of the general ability to set seed, but it is obvious that all the varieties are clearly self-incompatible, 90—95 % of the plants investigated being found below the limit of 5 % self set.

In Petkus rye 2879 self offspring plants representing 903 parent lines from I_0—I_4 have been studied (Table 2), and 24 I_0 lines are still represented in I_5 out of the 327 I_0 lines originally introduced. Although the data at hand do not lend themselves to a strict correlation analysis due to the unequal class intervals and the lack in most cases of the original individual seed set values in the offspring, the heritable nature of the ability to set self seed is quite clear. But it is apparent that offspring from both extreme parental classes may cover the whole range of variation. Of the offspring plants from the "lowest" parental class (0—5 % self-set) 80—90 % fall below the level of 20 % self-set; and 65—80 % of the offspring exceed this level in the "highest" parental class (50—100 % self-set).

TABLE 2. *Self seed set relations in parent/offspring plants (I_0/I_1—I_4/I_5) of diploid Petkus rye.*

Parent		Offspring plants									
Generation		Plant self seed set in per cent							Number of		Per cent plants	
		0	0	5	10	20	50	100	plants	families	above 20 % self-set	below 5 % self-set
I_0	0—5		194	217	14	18	59	63	565	296	21.6	72.7
I_1			77	100	6	9	16	9	217	100	11.5	81.6
I_2			27	51	10	4	5	4	101	53	8.9	77.2
I_3			20	25	5	4	3	6	63	25	14.3	71.4
I_4			11	15	6	3			35	16	0.0	74.3
I_0	5—10		22	74	16	6	3	2	123	20	4.1	78.0
I_1			36	78	15	8	9	2	148	34	7.4	77.0
I_2			8	9	9	5	1		32	11	3.1	53.1
I_3			5	20	8	9	8	6	56	22	25.0	44.6
I_4			2	13	1	9	3		28	6	10.7	53.6
I_0	10—20		20	66	18	7	12	20	143	9	22.4	60.1
I_1			23	29	7	15	10		84	13	11.9	61.9
I_2			7	18	16	11	12		64	20	18.8	39.1
I_3			2	10	6	10	17	1	46	16	39.1	26.1
I_4			4	10	9	5	7	2	37	13	24.3	37.8
I_0	20—50		2	13	7	6	2		30	1	(6.7)	(50.0)
I_1			29	41	23	37	86	10	226	43	42.5	31.0
I_2			18	41	26	24	52	15	176	51	38.1	33.5
I_3			4	4	5	8	94	48	163	41	87.1	4.9
I_4			5	10	8	18	34	6	81	22	49.4	18.5
I_0	50—100						6		6	1	(100.0)	(0.0)
I_1			11	4	5	33	72	36	161	36	67.1	9.3
I_2			11	9	5	12	47	33	117	22	68.4	17.1
I_3			3	1	—	6	23	19	52	16	80.8	7.7
I_4			13	4	7	19	63	19	125	16	65.6	13.6

At this point there is a remarkable tendency for the lowest parental class to yield proportionally more self offspring above 20 % self-set than does the next to lowest class (5—10 % self-set). It is also apparent that in the lowest parental class the proportion of offspring above 20 % self-set is decreased as the degree of inbreeding within the parental generation is increased. These tendencies are of considerable significance for assessing the factors involved in the origin of self-compatibility.

V. FACTORS INVOLVED IN THE ORIGIN OF SELF-COMPATIBILITY

1. New combinations of modifier factors versus new factors for self-compatibility

The origin of highly self-fertile offspring from self-incompatible parents may be imagined along two lines: (1) segregation of new specific combinations of modifier factors which act in the offspring plant to impair the pistil reaction to the self pollen, or (2) introduction by parental self gametes of a new factor conferring self-compatibility. It may be assumed that the frequency of parental pollen grains with eventual new factors for self-compatibility is independent of the frequency with which the remaining pollen will function on the self pistil. Upon an increase of the latter frequency, offspring plants with a

TABLE 3. *Comparison of self-fertility within offspring from parents of Petkus rye with low (0—5 % self-set) and moderately low (5—10 % self-set) self-fertility.*

(Patterns expressed in per cent of the total numbers of plants. The higher frequency values are in bold types.)

Parent plants		Offspring plants						
Generation		Plant self seed set in per cent						
		0	0	5	10	20	50	100
I_0	0—5	**34.3**	38.4	2.5	3.2	**10.4**	**11.2**	
I_1	0—5	**35.5**	46.1	2.8	4.1	**7.4**	**4.1**	
I_2	0—5	**26.7**	**50.5**	9.9	4.0	**4.9**	**4.0**	
I_3	0—5	**31.8**	**39.7**	7.9	6.3	4.8	9.5	
I_0	5—10	17.9	**60.2**	**18.0**	**4.9**	2.4	1.6	
I_1	5—10	24.3	**52.7**	**10.1**	**5.4**	6.1	1 4	
I_2	5—10	25.0	28.1	**28.1**	**15.7**	3.1	0.0	
I_3	5—10	8.9	35.7	**14.3**	**16.1**	**14.3**	**10.7**	

Heterogeneity test (performed on data in Table 2).

Parent generation	χ^2_c	P	Classes pooled
I_0	19.469	< 0.001	0—20; 20—100
I_1		0.105	„ „
I_2		0.255	„ „

TABLE 4. *Comparison of frequencies of "high" offspring (above 20 % self-set) obtained from I_0 parent plants of Petkus rye with low (0—5 % self-set) and moderately low (5—10 % self-set) self-fertility.*

Expected frequencies calculated from the observed frequencies of the 0—5 % series, assuming (A) independence, or (B) dependence, between occurrence of "high" and "low" offspring plants. (For explanation, see text!)

Parent plants		Offspring plants					
Generation	Self-set in %	Derivation of data	Plant self-set in per cent 0	20	100	Number of plants	families	% plants above 20 % self-set
I_0	0—5	Observed	443	122		565	296	21.6
	5—10	„	118	5		123	20	4.1
	5—10	Expected A	118.2	4.8		123.0		
	5—10	„ B	96.4	26.6		123.0		

Expected A $= \frac{122 \times 123}{565 \times 5.577} = 4.8$; Expected B $= \frac{122 \times 123}{565} = 26.6$.

new factor which confers self-compatibility will accordingly become relatively fewer, whereas the ratio of highly self-fertile offspring which are due to segregation of modifiers, will not be negatively affected.

The patterns of self-fertility in the offspring from "low" (0—5 % self-set) and moderately low (5—10 % self-set) parent plants are given in Table 3 for I_1—I_4, the I_5 generation being omitted owing to the low number of plants here. Except for the I_4 material, where selective trends may have acted in a disturbing way, it is found that, from the level of 20 % offspring seed set and upwards, the positive correlation between parent and offspring seed-setting ability is reversed. Thus, the "low" plants, at least in I_0, give significantly more offspring with self seed set above 20 %. In accordance with the place of this correlation reversal, the plants above 20 % self-set will be contrasted as "high" against the other plants. It is obvious that mere segregation of modifier factors cannot account alone for the "high" offspring plants, and that, at least in part, these plants must be due to the introduction of completely new factors for self-compatibility.

It is evident that the number of pollen grains or flowers selfed on the parent, or the number of offspring plants investigated, is of no relevance for an attempt to assess the parts to which these two alternatives are involved here in the origin of the "high" offspring. It will suffice to take into account the level of parental self seed set.

TABLE 5. *Total material of self seed set in I_1 plants obtained from parent plants with low (0—5 % self-set) or moderately low (5—10 % self-set) self-fertility.*

Parent		Offspring plants									
Variety		Plant self seed set in per cent							Number of		% plants above 20 % self-set
		0	0	5	10	20	50	100	plants	families	
Petkus rye	0—5	194	217	14	18	59	63		565	296	21.6
Storm-rye II	0—5	16	16	—	2	9	5		48	37	29.2
Star-rye	0—5	3	8	—	3	2	3		19	15	26.3
Brattingsborg rye	0—5	19	24	5	6	14	7		75	31	28.0
Brattingsborg × Schlaraffen rye	0—5	14	23	3	2	1	5		48	13	12.5
Petkus rye	5—10	22	74	16	6	3	2		123	20	4.1
Storm-rye II	5—10		2	—	1				3	1	(0.0)
Star-rye	5—10	1	1	1					3	1	(0.0)
Brattingsborg rye	5—10		12	1	1	—	1		15	3	6.7
Brattingsborg × Schlaraffen rye	5—10	10	14	4	4	2			34	2	5.9

"Low" and moderately low parents are compared in the I_1 offspring, where disturbances due to the inbreeding practised should be less significant (Table 4). The parental class means are 2.5 and 7.5 per cent, respectively, but especially for the lowest class a considerable error is introduced by the class grouping, because most seed set values closely approach the limit of 0 %. The means based on the individual parent values are 1.231 % and 6.865 %, respectively, the ratio between the means being 5.577. Strictly inverse relations between these parental means and the ratios of "high" offspring will indicate that the "high" offspring plants occur independently of the "low" plants and are due to the introduction of new factors for self-compatibility. Alternatively, an approach to a fixed ratio between "high" and "low" offspring plants will indicate that these categories are different expressions of the same genetical principle and predominantly due to segregation of factors modifying the perfection of the incompatibility reaction. It will be seen from Table 4 that there is no need to assume that "high" offspring plants should be due to other causes than the introduction of a new factor which confers self-compatibility.

The trends in the other rye varieties investigated, although with far

less extensive data, seem to be in close agreement with those revealed in Petkus rye (Table 5).

2. The origin of new factors for self-compatibility

The previous data indicated that "high" plants obtained from self-incompatible parents were predominantly due to a new factor which confers self-compatibility. This change from the original incompatibility factor to a factor for self-compatibility can be imagined as resulting from mutation or from recombination. A process effecting recombination will be detectable only in genetically heterogeneous material. New factors which are due to recombination should accordingly become rarer with a decrease of heterozygosity.

It was found in Table 2 that from parents with 0—5 % self-set "high" offspring were more rarely obtained within the more highly inbred parent series. This difference is more specifically illustrated in Table 6,

TABLE 6. *Comparison of patterns of self-fertility in Petkus rye in I_1 and I_2 offspring obtained from parent plants with low self-fertility (0—5 % self-set).*

Table	Parent Generation	Family category	Offspring plants: Plant self seed set in per cent 0	0	5	10	20	50	100	Number of plants	Number of families	Mean No. plants/family
a	I_0	Total material	194	217	14	18	59	63		565	296	1.91
	I_1	„ „	77	100	6	9	16	9		217	100	2.17
b	I_0	Families with "high" plants	50	44	5	6	59	63		227	93	2.44
	I_1	Families with "high" plants	7	12	1	4	16	9		49	21	2.23
c	I_0	Families without "high" plants	144	173	9	12				338	203	1.67
	I_1	Families without "high" plants	70	88	5	5				168	78	2.15

Difference between I_1 and I_2 offspring.

Table	χ^2_c	P	Classes pooled
a	10.492	0.01—0.001	0—5; 5—20; 20—100
b		0.425	0—20; 20—100
c		0.540	0—5; 5—20

TABLE 7. *Offspring self-set in different years for I_0 and I_1 parents of Petkus rye with self-set below 5 %.*

Parent		Offspring plants									
Generation	Year	Plant self seed set in per cent							Number of		% plants above 20 % self-set
		0	0	5	10	20	50	100	plants	families	
I_0	1920	50	41	—	2	13	16		122	56	23.8
	1921	15	24	3	3	7	11		63	31	28.6
	1922	66	75	5	7	26	20		199	118	23.1
	1923	2	3	—	1				6	4	—
	1924	8	5	—	—	3			16	11	18.8
	1925	4	19	4	1	1	2		31	15	9.7
	1926	49	50	2	4	9	14		128	61	18.0
I_1	1920	—	—	—	—	—	—		—	—	—
	1921	1	6	—	—	—	2		9	7	22.2
	1922	18	46	3	4	10	7		88	55	19.3
	1923	34	26	2	2	4			68	18	5.9
	1924	5	4	1	2	1			13	5	7.7
	1925		1	—	1				2	1	—
	1926	19	17	—	—	1			37	14	2.7

which concentrates upon the I_1 and I_2 offspring groups where selective disturbances in connection with the inbreeding practised should be less prominent.

The significance in Table 6 a of the higher rate of "high" I_1 plants for assessing the presence of factors for self-compatibility as due, at least in part, to recombination, should not be overestimated, seeing that several sources of error may influence the rates of "high" offspring plants. One of these sources of error is immediately apparent. Rye is an annual plant, and parents and offspring were not selfed in the same year; therefore, strictly comparable I_1 and I_2 data are not available. The influence of the seasonal conditions is studied in Table 7. At least for the I_1 offspring there are no clear indications of essential disturbances in the pattern of self-set which may be due to differing seasons. The inbred I_2 plants may be more sensitive to the season, but the data are somewhat meagre.

Next, the weakening of the plants, as the degree of inbreeding is increased, is important, and this applies especially to self seed set, because it is the product of pollen production and female efficiency. The level of self seed set may not be a true expression of the level of

self-compatibility, and the weakening of the plants as they are more inbred, may account for the whole decrease in rate of "high" self offspring.

The patterns of self seed set are, therefore, studied in two separate parts from the total material of Table 6 a. Offspring families counting at least one "high" plant are separated from those families where no "high" plants were obtained (Table 6 b and 6 c). The argument is that agreement in the patterns of offspring self-set between the I_0 and I_1 series in both of these tables will strongly indicate that a reduced frequency of new factors for self-compatibility contributes to the difference displayed by the total materials in Table 6 a. On the other hand, a difference between the series in either or both of the tables will permit no definite conclusion and may equally well indicate a weakening effect of inbreeding.

Both tables reveal a close agreement between the series. Owing to the low mean number of plants investigated per family — less than 2.5 — the revealed agreement between the series in Table 6 b should not be overestimated. This caution is the more necessary, as the I_1 series gave rise to only 25 "high" offspring plants — nearly the minimum for the 22 selected offspring families — and the I_0 series, in spite of its higher mean number of plants per offspring family, gave the higher rate of "high" offspring plants (53.8 % against 51.0 %). More conclusive is the close agreement between the I_0 and I_1 series in Table 6 c. Here the series involve the majority of the plants, and the region up to 20 % self-set is covered. It is concluded that a real reduction in the rate of new factors for self-compatibility is indicated for the I_1 parent series.

At this point regard must be paid to the fact that plants which are completely sterile upon selfing are more frequent among I_1 plants than in the I_0 group. In the group of plants below 5 % self-set, 20 % of the I_0 plants and 39.1 % of the I_1 plants set no self seed. This difference may work in opposite directions. On the one hand, the sterile I_1 group may contain particularly weak plants, the elimination of which will keep up the ratio of "high" plants in the I_2 offspring. On the other hand, it is evident that the lower number of pollen grains tested against the incompatibility sieve in the weaker I_1 parent series may be of significance. It was shown in Table 4 that "low" parents gave a higher proportion of "high" offspring than did parents with moderate self-set, and it may be argued that part of the sterile I_1 plants correspond to I_0 plants with an especially high proportion of "high" offspring.

To test this assumption the ratio of "high" offspring for I_0 parents

TABLE 8. *Pattern of self-fertility in I_1 offspring from parent plants of Petkus rye with extremely low (0—1% self-set) or low (1—5 % self-set) self-fertility.*

Parent		Offspring plants									
Generation		Plant self seed set in per cent							Number of		% plants above 20 % self-set
		0	0	5	10	20	50	100	plants	families	
I_0	0—1	77	56	7	9	26	26		201	151	25.9
	1—5	117	161	7	9	33	37		364	145	19.2

with self-set below 1 % is contrasted to the corresponding ratio for I_0 parents setting 1—5 % self seed (Table 8). The difference between the ratios (25.9 % for the lower, and 19.2 % for the higher parent class) corresponds to $\chi^2_c=3.160$ (P=0.1—0.05; classes 0—20 % and 20—100 %, pooled). The difference is less marked than expected from the parental mean levels of self seed set, even when the latter are based on the individual parent values (0.517 % and 1.974 %, respectively, the ratio between the means here being 3.82). Probably a change in the biotypic proportions effects this deviation from the strictly inverse relation between parental self-set and ratio of "high" offspring, so beautifully revealed when the I_0 parental classes with 0—5 % and 5—10 % self-set were contrasted, The parent class with 0—1 % self-set may, thus, contain a higher proportion of plants less liable to throw new factors for self-compatibility.

Attention must now be drawn to the actual mean levels of self seed set in the I_0 and I_1 parental series. The means based on the individual parent values are 1.231 % and 1.959 %, respectively. It is obvious that such a difference between the means can account for a considerable part of the decrease of the ratio of "high" offspring plants for the I_1 parent series. Part of the difference between the means, however, is due to the increase of the discontinuity between 0 % and >0 % self-set in connection with the decrease of the number of selfed flowers in the inbred I_1 series. It is possible, however, to demonstrate in another way that the error introduced by the differences in mean levels can be neglected. As already seen from Table 8, the I_0 parental class with 1—5 % self-set has a mean level of 1.974 %, almost identical to the I_1 parent mean 1.959 %, but the ratio of "high" I_1 offspring plants is here 19.2 %. The difference between the ratios 19.2 % and 11.5 %, the latter

TABLE 9. *Self seed set relations in three successive generations of inbreeding ($I_0/I_1/I_2$). Pattern of self-fertility in I_2 offspring plants from parents with 0—5 % self-set but derived from I_0 plants of various self-fertility.*

Grandparent	Parent	Offspring plants									
		Plant self seed set in per cent							Number of		% plants above 20 % self-set
		0	0	5	10	20	50	100	plants	families	
0—5	0—5	35	45	4	6	10	1		101	47	10.9
5—10	0—5	10	22	—	1	3	3		39	20	15.4
10—50	0—5	32	33	2	2	3	5		77	33	10.4
	Total	77	100	6	9	16	9		217	100	11.5

obtained for the I_1 parent series, corresponds to $\chi^2_c = 5.358$ ($P = 0.05—0.02$; classes 0—20 % and 20—100 %, pooled).

A possible source of error is the heterogenic derivation of the I_1 parental class with 0—5 % self-set, as suggested from Table 2. It may be seen from Table 9, however, that the comparison with the corresponding I_0 parent class is not seriously disturbed.

Also within the Brattingsborg rye "high" offspring from parents with 0—5 % self-set are less frequent in the I_2 than in the I_1 series (Table 10). It should be observed here that within neither of the two varieties is such a decrease observed for the parental class with 5—10 % self-set. The data have been considered too meagre, however, to inquire into this deviation.

The analysis of the reduced ratio of "high" offspring from the inbred I_1 parents would have been safer and less complicated, had pollination

TABLE 10. *Self seed set relations in parent/offspring plants ($I_0/I_1—I_1/I_2$) of diploid Brattingsborg rye.*

Parent		Offspring plants										
Generation		Plant self seed set in per cent							Number of		Per cent plants	
		0	0	5	10	20	50	100	plants	families	above 20 % self-set	below 5 % self-set
I_0	0—5	19	24	5	6	14	7		75	31	28.0	57.3
I_1	0—5	20	29	5	1	6	4		65	21	15.4	75.4
I_0	5—10		12	1	1	—	1		15	3	6.7	80.0
I_1	5—10	4	17	9	3	8	5		46	4	28.3	45.7

conditions and female efficiency been known for the selfed plants. Nevertheless, clear indications are obtained that general weakness, accompanying inbreeding, cannot account for the whole reduction of the ratio of the "high" plants in the I_s offspring. Obviously, *new factors for self-compatibility are produced at a lower rate in the I_1 parents than in the I_0 parents.*

The rate of "high" offspring per selfed floret from I_0 plants below 5 % self-set in the present Petkus material is calculated to 0.00168. This figure is obtained as the product of the rate of "high" I_1 offspring, which is 0.216, by the rate of self seeds per selfed I_0 floret (value calculated to 0.0078 and obtained for 264 I_0 plants where data on numbers of selfed ears and individual seed set were available). The average number of pollen grains on the selfed stigma is not known, but on the assumption of a number varying from 100—10000 the rate of self-compatible pollen grains from I_0 plants below 5 % self-set would lie in the region between 10^{-5} and 10^{-7}. This frequency seems to be high enough to be consistent with the assumption, not only of strict mutations, but also of recombinations within the incompatibility gene, both being able to bring about factor changes which lead to self-compatibility.

VI. DISCUSSION

The present data give no direct indications as to the system of self-incompatibility operating in the investigated varieties of diploid rye, but Petkus rye being the progenitor of Steel-rye, investigated by LUNDQVIST (1954, 1956), the same two-loci system is most probably operating here, too. There is a remarkable agreement in general trends between the two varieties, although some of them were less obvious and not noticed in the data of Steel-rye (LUNDQVIST, 1958). The present trends may be summed up as follows:

Self-sterile plants were predominant in the outbred populations. The ability to set self seed was clearly heritable, but self offspring with high self-set were recovered from parents of both extremes. Proportionally more self offspring above 20 % self-set were recovered from the lowest than from the next to lowest parental class of self-set. From this inverse relation between parental level of self-set and the rate of "high" self offspring it could be concluded that segregation of modifier factors affecting the perfection of the incompatibility reaction was of no conse-

quence. New factors conferring self-compatibility and directly involving the incompatibility loci had to be introduced.

Some light was thrown upon the production of such new factors by the observation that the rate of "high" self offspring from the "lowest" parental class was decreased as the parents were more inbred. A consideration of the variables introduced here by (1) influence of differing seasons on the annual rye, (2) inbreeding depression reducing general fertility, 3) the lower number of pollen grains tested in the weaker inbred plants, and (4) the heterogenic derivation of an inbred parental group, led to the conclusion that the rate of "high" self offspring was also directly influenced by the rate of parental heterozygosity.

The decreased rate of new factors for self-compatibility which accompanies the decrease in rate of heterozygosity may be taken to indicate that at least in part the new factors are due to recombination. BREWBAKER and SHAPIRO (1959) discussing similar data in *Petunia* from an experiment especially designed to test this question, point out that either equal crossing-over or gene conversion will be expected to produce self-compatible pollen only in S heterozygotes, whereas unequal crossing-over may produce such pollen in both homozygous and heterozygous plants (duplicative pollen with competition interaction in the heterozygotes, and deficient pollen in both heterozygotes and homozygotes).

It should be noted here that the possibility of unequal crossing-over can be ruled out in the present material as a cause of the lower rate of new factors for self-compatiblity in the more homozygous individuals. Pollen deficient in an incompatibility factor will have equal possibilities of being formed through unequal crossing-over in both homozygotes and heterozygotes, but, judging from the behaviour of the diploid pollen (LUNDQVIST, 1957, 1958), the reciprocal product with two *different* incompatibility alleles on the same chromosome (which can only be formed in the heterozygote) will show no competition interaction in rye.

If one assumes that equal recombination operates to produce self-compatibility, two conclusions will follow:

(1) The chromosome segments carried by the recombinant pollen are all represented in the self pistil, but their juxtaposition is not identical. The absence of an incompatibility reaction between pistil and self recombinant pollen thus indicates the *disorganization of a functional system* and that the incompatibility reaction is dependent on juxtaposition of the segments in question.

(2) In the individual resulting from the fertilization by such a re-

combinant pollen, identical chromosome segments have identical juxtaposition in pistil and (non-recombinant) pollen. The absence of an incompatibility inhibition of self pollen at this stage indicates that the postulated disorganization of the functional system has led, either to the lack of formation of a reacting substance, or to lack of reaction between substances in pistil and pollen.

Lack of formation of a reacting substance after recombination would indicate considerable differences betwen the specific regions of the two incompatibility alleles. In view of the successful co-operation between the multiallelic S and Z loci in the elaboration of the incompatibility S—Z specificities within the two-loci incompatibility system in rye, only small differences between the alleles within each of these loci should be expected (LUNDQVIST, 1958). With the present incompatibility system equal recombination is more likely to lead to new specificities, and self-compatibility would rather indicate the lack of co-ordination between the substances formed in pistil and pollen.

What structure of the incompatibility locus is implied by a position effect leading to self-compatibility? It has been suggested (DARLINGTON and MATHER, 1949, p. 332) that the incompatibility S gene has two parts: a carrier necessary for the activity of the substances and a specificity imprinter common to pollen and pistil. As pointed out by LEWIS (1958), self-compatibility must with this interpretation require a change in the carrier segment, as a change in the specificity segment would lead to an active and changed specificity. It seems obvious that equal recombination within the carrier segment will lead to nothing new; otherwise it seems difficult to conceive the need of a special specificity imprinter for segments which by themselves could already be specific. Self-compatibility due to equal recombination is not compatible here with this bipartite structure of the incompatibility locus.

The situation will be changed if one assumes, as suggested by LEWIS (1954) and CROWE (1955), that the specificity imprinter is *not* common to pollen and pistil, the incompatibility locus including two specific regions that determine specificity, one for the pistil and the other for the pollen. For this system to ensure self-incompatibility the particular complementary pollen and pistil regions have to travel on the same chromosome. Recombination within the incompatibility locus leading to separation of these two complementary regions will also lead to disorganization of this mechanical system, but probably only recombination within the "pollen region" will lead to pollen which is immediately compatible on the self pistil of a self-incompatible plant.

It should be noted here that on this scheme the observation of plants with low self-set in the self offspring of self-compatible plants might find an explanation. Such deviating plants were not rarely found in the inbred populations of Steel-rye, and a high proportion of them were demonstrated to be due to inbreeding depression (LUNDQVIST, 1958). On the present scheme recombination within the "pistil region" might restore the co-ordination between the pollen and pistil specificities which was once disturbed by a previous equivalent recombination within the "pollen region" of the incompatibility locus. Thus, the origin of a functional new incompatibility allele might by conceived, and self-incompatibility would be restored. The simultaneous coincidence of two such recombinations in both specificity regions must be an extremely improbable event. Deviating plants with low self-set were also found in the present material, but in the absence of information as to the influence on general fertility of the inbreeding practised, their origin is obscure.

Juxtaposition would thus be required at one part of the chain of synthesis of the incompatibility substances, whereas the final reacting products result from the complementary co-operation between the independently inherited loci S and Z. It does not seem profitable, however, to speculate more on the nature of these loci and the substances they form, before the presumed process of recombination has been studied in more detail and more light shed on the structure of the two incompatibility loci. More adequate material is now being studied.

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SUMMARY

The ability to set seed upon enforced self-pollination by means of bagging was studied in four diploid commercial varieties of rye and one hybrid population. Petkus rye was the main material with more than 3600 bagged plants ranging from I_0—I_5.

Self-sterile plants were predominant in the outbred populations. The ability to set self seed was clearly heritable, but self offspring with high self-set were recovered from parents of both extremes. Proportionally more self offspring above 20 % self-set were recovered from the "lowest" than from the next to lowest parental class of self-set. From this inverse relation between parental level of self-set and rate of "high" offspring

it could be concluded that segregation of modifier factors affecting the perfection of the incompatibilty reaction was of no consequence. New factors conferring self-compatibility and directly involving the incompatibility loci had to be introduced.

Within the "lowest" parental class the rate of self offspring with high self-set showed a decrease with an increase of inbreeding. It was made probable that general weakness from inbreeding could not account for the whole of this reduction, and that the lower rate of new factors for self-compatibility in the less heterozygous series indicated that, at least in part, these factors were due to recombination.

The implications of recombination within an incompatibility locus operating to produce factors for self-compatibility are discussed. With the two-loci system which operates in rye, equal recombination is not considered to lead to lack of formation of incompatibility substances but to new specificities, and self-compatibility would rather indicate the lack of co-ordination between the substances formed in pistil and pollen. Within the incompatibility locus two regions that determine specificity are made probable, one for the pollen, the other for the pistil. Recombination within the incompatibility locus leading to separation of these two complementary regions will also lead to disorganization of this system.

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SPONTANEOUS AND INDUCED MUTATIONS OF BARLEY FOR THE REACTION TO MILDEW

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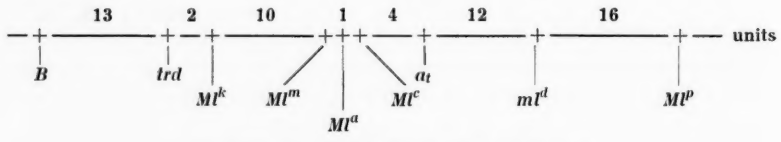
THE genetics of resistance in barley to the two pathogenic races of mildew (*Erysiphe graminis* DC) hitherto found in Argentina has been already studied (FAVRET, 1949, 1953, 1956) in about twenty varieties. Summarizing the results, fourteen factors for resistance are distributed in nine loci located on only one chromosome. This chromosome corresponds to the second linkage group and very probably to that named as *a* or *5*, the smallest chromosome of barley without a satellite (BURNHAM and HAGBERG, 1956). Other loci for resistance to other races of mildew have been located on the same chromosome (SCHALLER and BRIGGS, 1955, HIURA *et al.*, 1956). Of the eighteen factors for resistance distributed on 14 loci, at least seventeen are in chromosome *5* and one in chromosome *4*, corresponding to the fourth linkage group.

According to SCHALLER and BRIGGS (1955), SMITH (1951) and my own data, the second linkage group would show diagramatically the following sequence:



plus Ml^r, Ml^{go}, Ml^{mw}, Ml^{mu}, Ml^{3576}, ml^w, ml^s.

One of the loci, Ml^p, has at least 3 or 4 alleles for resistance. Three other loci form a genic complex (FAVRET, 1953) being very closely linked and involving six or perhaps seven different alleles or pseudo-

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alleles for resistance. In its totality this long genetical segment, about 45—50 units, represents apparently a very good example of what KAPLAN (1952) called "an isophenic segment".

The character of immunity or resistance to mildew has proved to be very useful for genetical studies. Since it has selective advantage, it will be no doubt valuable for evolution experiments. For practical purposes, it represents a good example of a way to improve our crops in the struggle against diseases. It is interesting for all these studies to know how frequently the genes controlling mildew reaction mutate under different conditions. Thus, some results on this problem are reported here.

MATERIAL AND METHODS

The results here presented have been obtained in studies from 1950 until 1958. During these studies ten varieties of barley have been used, four of them being susceptible to the *Arg. 2* race and the other six immune or resistant; but three of them have been extensively studied. They are Maltería Heda (susceptible), Engledow India and Monte Cristo (immunes). FAVRET and GODECK (1959) have not found, studying 36 barley varieties, any statistically significant difference for spontaneous mutability. Then, it seems to be justified to pool the data coming from the susceptible varieties on one side and the immune and resistant varieties on another.

The treatments with gamma-rays or neutrons were performed at the Comisión Nacional de Energía Atómica, at Buenos Aires. The gamma-rays were produced by a $Cobalt^{60}$ source delivering 10 r/min. The fast neutrons were produced in an accelerator working at a flux of 10^6 V. and 135 μA., giving .47, .79 and 1.50 $N \times 10^{12}/cm^2/hour$. Fifteen treatment were carried out on dormant seeds with a moisture content of about 11 %.

The chemical treatments were made by soaking the seeds in ethylene oxide or formaldehyde for variable times and concentrations. Finishing the treatments, the seeds were washed thoroughly in tap water and immediately sown in the field. As some treatments last for 24 hours, it is possible that the seeds have started to germinate.

The treated (M_1) and control plants were grown isolated from other barley material and harvested individually, taking 4 spikes from each plant. All the M_2 seedlings have been grown under greenhouse conditions and infected with a clone of the race *Arg. 2* of mildew (FAVRET, 1947) which has been maintained asexually without any apparent change in

its pathogenicity. The chlorophyll mutants have been recorded following GUSTAFSSON's designations (1940).

As it is usual, mutations were scored only when the progeny from one of the four spikes coming from one plant was segregating. Those segregating in all the 4 spikes for the same phenotypic character, were discarded from the counts since they were not chimaeras and consequently not produced during the M_1 generation. As an average, 15 seedlings were grown from each M_1 spike. Each spike progeny carrying a mutation was transplanted and isolated, studying at least one new generation, in order to determine its type of segregation, dominance, etc. In some cases one plant from each M_1 spike progeny was analysed in M_2, the mutation rates calculated in both generations being about the same.

The spontaneous mutation rate is assigned to a generation basis, but we must be aware of the fact that in the method here used, a difference exists between dominant and recessive mutants. The first ones will be detected in the next generation no matter the moment, during the entire plant cycle, when they have been produced. The recessive mutations, on the other side, can only be checked when produced before the separation of the male and female tissues in the spike primordium; later on, they will be masked by the normal dominant allele. This is of no importance in mutagenic treatments since the mutation occurs probably at the time the treatment is performed or within the very next cellular divisions.

RESULTS AND CONCLUSIONS

The pooled data gathered from all the experiments, except neutrons treatments, are presented in Table 1. In the last column the mutation rate for reaction to mildew (called *m*) has been divided by the rate of chlorophyll mutation (*c*). This permits a comparison of different treatments because it has been demonstrated frequently that chlorophyll mutations are good indicators for measuring mutagenic effectiveness in barley.

In some experiments, certain chemicals have induced a proportion of mutants different from that obtained in spontaneous mutability. For example, nebularine (EHRENBERG *et al.*, 1956), formaldehyde (FAVRET and RODRÍGUEZ, 1957) and mustard gas (MAC KEY, 1954) induce *viridis* types mutations but not *albina* ones. In such cases, it would be better to relate the other kinds of mutations to the *viridis* chlorophyll mutants.

Mutations to a higher level of resistance have not been obtained

TABLE 1. *Pooled data on spontaneous and induced mutations for reaction to mildew and chlorophyll deficiencies.*

Genetical change	Treatment	Number spike progenies	Mutations for reaction to mildew (m)	Chlorophyll mutations (c)	m:c ratio
Susceptibility to resistance [1]	None	38.141	0	22	—
	Gamma-rays	8.531	14	213	.065
	Ethylene oxide	9.251	0	41	—
	Formaldehyde	15.849	1	44	.022
Resistance to susceptibility [2]	None	23.377	3	13	.230
	Gamma-rays	3.783	1	147	.006
	Ethylene oxide	5.612	2	57	.035

[1] Susceptible barleys: Maltería Heda— Trebi— La Previsión 19— Edda.

[2] Resistant barleys: Engledow India — Monte Cristo— *Hordeum spontaneum nigrum*— Psaknon— Multan— Spiti.

spontaneously, though a great number of progenies were tested. On the other hand, a lower level of resistance often appears in the control series and its frequency is about one fourth of the chlorophyll mutants (*m:c* ratio=.230). A higher level of resistance has been obtained following mutagenic treatments of gamma-rays, formaldehyde (only once) and fast neutrons (see Table 3). Negative results have been obtained with ethylene oxide, in spite of a very marked increase in the rate for chlorophyll mutations.

Formaldehyde is only half as strong as the ionizing radiations, as shown by the *m:c* ratios calculated on *viridis* mutations, in the treatments of Maltería Heda barley:

gamma-rays ..... .050
neutrons ..... .050
formaldehyde ..... .025

The treatments for increasing the mutation rate have been very ineffective in producing at lower level of resistance. A slight increase of mutations for reduced resistance has been obtained, especially with ethylene oxide. However, this increase was not statistically significant.

In Table 2 are presented the effects of the gamma-rays on the Maltería Heda barley, in terms of the mutation rate per spike and the mutation rate per spike per röntgen for chlorophyll and resistance to mildew mutants, thus the *m:c* ratio. In Figure 1, the curves for frequencies for

TABLE 2. *Frequencies of resistance to mildew and chlorophyll mutations obtained with gamma-rays treatments in the barley variety Maltería Heda.*

Dose (r)	Spike progenies	Mutations for resistance (m)	Cloroph. mutations (c)	m/spike $\times 10^{-4}$	c/spike $\times 10^{-4}$	m/spike/r $\times 10^{-8}$	c/spike/r $\times 10^{-8}$	m:c ratio
3.000	713	0	5	0.0	70.0	0.0	233.3	.—
10.000	3.649	3	83	8.2	227.4	8.2	227.4	.03
20.000	651	3	30	46.0	460.0	23.0	230.0	.10
30.000	551	8	33	145.1	598.0	48.3	199.0	.24
Total	5.564	14	151	25.1	274.8	—	—	.099

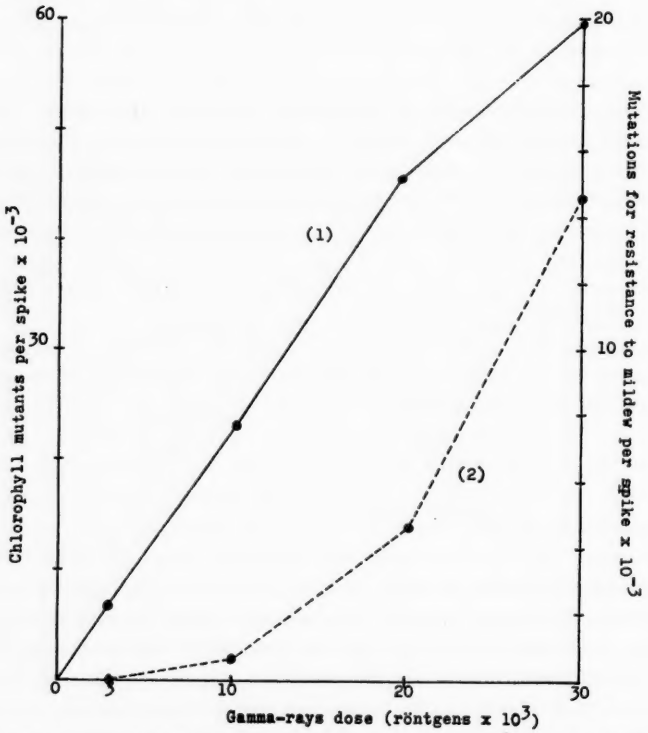

Fig. 1. Frequency of chlorophyll mutations (1) and mutations for resistance to mildew (2) plotted against gamma-rays dose.

TABLE 3. *Chlorophyll and resistance to mildew mutations induced by gamma-rays and fast neutrons in the barley variety Malteria Heda.*

	Fast neutrons	Gamma-rays
Spike progenies	1.406	5.564
Mutations to resistance (m)	5	14
Chlorophyll mutations (c)	50	151
m/spike$\times 10^{-4}$	35.5	25.1
c/spike$\times 10^{-4}$	355.6	275.8
m:c ratio	.10	.099

borth kinds of mutations are represented. For equating values we have increased the mutation rate scale for resistance to mildew by a factor of 3.

It is observed a different function according to the character studied. Mutation frequency increases exponentially for resistance to mildew (by a factor of 2) while chlorophyll mutants follow a straight line. Thus, the highest value of the *m:c* ratio (.24) was obtained at the maximum dose of 30,000 r.

A comparison of the effectiveness of neutrons and gamma-rays on a single variety, is presented in Table 3. This is based principally on the *m:c* ratio, since different doses have been used in each case. Both mutagens are about equally effective, with a *m:c* ratio of .1).

In Table 4 are summarized the data of Table 1, expressed in mutation rate per spike$\times 10^{-4}$, thus showing the relative increase for each one of the four characters studied. The various mutagens produce some differences in mutation spectra. Ionizing radiations induce mutants of all classes, including the somewhat doubtful mutations from resistance to susceptibility. Ethylene oxide, on the other hand, has not induced resistant mutants and formaldehyde is unable to induce *albina* mutations.

TABLE 4. *Mutation rate per spike. Data of Table 1* $\times 10^{-4}$.

Mutations		Susceptible to resistant	Resistant to susceptible	Chlorophyll mutations		
				Total	Albina	Viridis
Spontaneous		0.0	1.3	5.7	2.7	3.0
Induced	Gamma-rays	16.4	2.6	292.3	142.0	150.3
	Ethylene oxide	0.0	3.5	65.9	31.5	34.4
	Formaldehyde	0.6	—	27.7	2.8	24.9

Ethylene oxide seems to be a stronger mutagen than formaldehyde but less effective than ionizing radiations.

The chief result of these studies is that the process of obtaining mutations for a changed reaction to mildew, is different according to the sense of that change. Spontaneous mutations for a lower level of resistance are relatively easy to obtain but possibly none can be attributed to the mutagens used. On the other hand, no spontaneous mutations to a higher level of resistance have occurred but they are obtained after treatments with strong mutagens. The differential spontaneous behaviour is even more striking if we recall that the higher level of resistance is sought in varieties carrying at least as many as 9 factors for susceptibility while the six resistant and immune varieties used in these experiments have only one locus or perhaps two very closely linked loci for resistance. The fact that the genes for resistance are dominant or semi-dominant may be of some importance in this respect. Mutations from the dominant to the recessive are usually easier to produce than vice-versa.

Apparently, to obtain a higher level of resistance, it is necessary to cause a drastic change in the chromosome and this can be achieved with mutagens capable of chromosome breakage. This conclusion is supported by the fact that the mutation rate for resistance to mildew follows an exponential curve with increasing dose. On the contrary, changes from resistance to susceptibility are obtained under natural conditions and possibly as a consequence of minor changes in the locus or recombinations of small segments of chromosomes. Mutagens such as gamma-rays or neutrons would be less able to affect the chromosomes in this way.

The difference between ethylene oxide and ionizing radiations in the production of mutations for resistance to mildew is very interesting but not surprising. The more careful the studies are undertaken, the more differences are found between the genetic effect of the ionizing radiations and the so-called radiomimetic chemicals.

Similarity in the mutagenic capacity of several mutagens is also difficult to prove when conclusions are drawn from the averaged effect upon many different loci. HAGBERG *et al.* (1958) have pointed out from studies of the "erectoides" mutants of barley, that it is necessary to analyse the different loci separately because some of them react differently depending upon the mutagen used. Thus, locus *a* mutates more frequently following gamma-rays or X-rays than neutron treatments, and locus *c* mutates often when a chromosome breakage point occurs at

the same place or nearby. Consequently the similar effect found in our experiments with fast neutrons and gamma-rays could be only apparent. It is necessary to develop new technics using a "per locus basis" if the mutation mechanism in plants is to be understood.

As the loci for resistance to mildew are physiologically identified by very specific and constant norms of reaction, comparable only to serological reactions, it is already evident that they can play an important role in understanding the process of mutation.

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SUMMARY

The mutation rate has been studied in barley for a changed reaction to mildew (*Erysiphe graminis* DC) using immune, resistant and susceptible varieties. At the same time, chlorophyll mutation rate has been recorded and a ratio between both frequencies has been suggested for measuring the mutagenic activity.

Mutations for immunity or resistance to mildew have been obtained in treatments with gamma-rays, fast neutrons and formaldehyde. None was obtained in control series or in ethylene oxide treatments. Plotted against dose, mutation frequency for resistance to mildew has shown an exponential increase, while chlorophyll mutation frequency increased linearly.

Mutations for susceptibility to mildew occurred spontaneously and only a slight increase in their frequency, not statistically significant, was obtained using ethylene oxide or gamma-rays.

The several mutagens used in these studies have shown different mutation spectra. Formaldehyde was unable to induce *albina* mutants, while ethylene oxide did not produce mutants resistant to mildew. This chemical is a stronger mutagen than formaldehyde but weaker than ionizing radiations.

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SOME RELATIONS BETWEEN NUMBER OF SEEDS PER POD, SEED SIZE AND OIL CONTENT AND THE EFFECTS OF SELECTION FOR THESE CHARACTERS IN BRASSICA AND SINAPIS

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SEVERAL investigations have been made concerning correlations between different characters within individual plants of rape (*e. g.* MANDEKIC 1912, BAUMANN 1918, STOLLE 1954, SCHRIMPF 1954, RIVES 1957). Some of the correlations demonstrated are of minor interest — *e. g.* the positive correlation between the weight of the empty, threshed pods and the seed weight of the plant; others are rather self-evident, *e. g.* the positive correlation between number of pods and seed weight (MANDEKIC 1912), but others are of great interest in practical breeding work. Among these may be mentioned the positive correlations between seed yield of the plant and number (MANDEKIC 1912, BAUMANN 1918, STOLLE 1954) or weight (MORICE 1956) of seeds per pod and the one between number of seeds per pod and pod length (MANDEKIC 1912, RIVES 1957). As an example of a practically important negative correlation may be mentioned the decrease in hardiness which follows increased stem height in the autumn (TORSSELL 1955).

From the breeders point of view, however, the correlations within the plant are hardly as important as the correlations between the characters of the plant and those of its progeny. These correlations offer a possibility to judge the relative importance in variability of modifications and genetic differences. The determinations of the regression of progeny upon mother plant is one of the possibilities to estimate the heritability of a character (LUSH 1949). Unfortunately there are no direct studies on the effects of selection in *Brassica* and *Sinapis*. The present paper deals with the effects of selection after number of seeds

per pod and after oil content and also with the correlations between these characters and some others within the plant.

I. MATERIALS AND METHODS

The investigations have been made on the breeding material of the Swedish Seed Association of rape (*Brassica napus* L. ssp. *oleifera* (METZG.) SINSK.), turnip rape (*B. campestris* L. ssp. *oleifera* (METZG.) SINSK.) and white mustard (*Sinapis alba* L.). For studies of the variability and correlations of different characters plants and plant progenies from the following varieties or crosses have been used:

Winter rape:

1. Sv 48/25 from Matador
2. Lembke's × Dippe's rape
3. Sv 48/25 × Dwarf Essex
4. Janetzki's × Dippe's
5. Matador × Vestal
6. Sv 55/1069 (from artificial rape 5) × Matador

Summer rape:

1. Sv 44/107 from X-ray treated Regina
2. [Sv 44/107 × (Regina × Lembke's winter)] × Liho
3. Regina summer rape × Lembke's winter rape
4. Sv 55/102 from Regina summer rape × Lempke's winter rape

Winter turnip rape:

1. Duro

White mustard:

1. X-ray treated Svalöf white mustard
2. Czechoslovakian white mustard
3. X-ray treated Primex white mustard
4. Sv 52/262 from Sv 44/303 from X-ray treated Svalöf white musard.

Other material used will be identified in connection with the experiments where it has been used.

Of the species studied rape is spontaneously self-fertilizing to about two thirds while turnip rape and white mustard are totally cross-fertilized (OLSSON 1952, OLSSON and PERSSON 1958). Openly flowering material has been used.

TABLE 1. *Coefficients of variation for seed yield per plant, number of seeds per pod, 1000 grain weight and oil content in plants of rape and white mustard.*

Character		Winter rape			Summer rape		White mustard				Mean acc. to STOLLE (1954)
		3	5	6	2	4	Unselected Primex			4	
							1955	1956	1957		
		n = 169	n = 110	n = 88	n = 120	n = 103	n = 100	n = 100	n = 100	n = 76	
Seed yield (g.)	$\bar{x}$	17.79	16.59	6.39	10.77	9.35	9.63	12.56	17.18	13.84	—
	v	41.4[1])	32.8[1])	36.1[1])	37.8[1])	49.0[1])	64.6	66.1	52.3	28.4[1])	71.3
Number of	$\bar{x}$	—	25.60	25.59	25.32	29.85	5.17	5.74	5.87	—	—
seeds per pod	v	—	16.1[1])	12.5[1])	13.5[1])	10.6[1])	12.8	13.5	12.7	—	30.0
1000 grain	$\bar{x}$	—	5.10	4.77	4.45	4.57	8.59	8.65	8.08	—	—
weight (g:)	v	—	9.2	8.6	12.0	11.2	13.8	14.7	13.3	—	17.1
Oil content %	$\bar{x}$	50.61	46.61	46.44	43.36	44.46	27.75	30.30	30.87	32.10	—
	v	4.0	3.9	3.8	5.6	3.6	7.7	8.4	7.4	8.2	6.7

[1] Selected in the field for seed yield and length of pod.

During routine harvest of individual plants "poor" plants are discarded in the field and only well developed plants with "good" pods are harvested and threshed. After threshing, plants with a yield of less than 4 grams have further been discarded since the amount of seed was not sufficient both for chemical analysis and for sowing. In regard to number of seeds per pods and seed weight per plant the material here used is, therefore, not quite adequate for a purely theoretical study of the effect of selection; on the other hand it deals with that part of the total material which is of real interest to the breeder, and the results may be used for conclusions regarding breeding technique. As to oil content and seed size (determined as weight of 1000 seeds) no selections are made in the field. In some cases the progeny of all plants with a known oil content has been sown, in others mostly the extreme classes have been used, those with intermediate oil contents have been partly excluded. This latter restriction of the material makes it possible with some reduction of work to determine the regression of the progeny upon the mother and thus to study the effect of selection, but the correlation obtained is not typical of the population (FISCHER 1938, p. 183).

In order to give an idea about the degree of variation in the material after the selections described, Table 1 gives the mean values ($\bar{x}$) and coefficients of variation (v) for some of the populations used.

The number of seeds per pod in rape and turnip rape has been determined on ten pods from the terminal inflorescence; in mustard with a lower number of seeds 20 pods have been used. In determining 1000 grain weight of individual plants 1×100 seeds have been weighed, in progeny plots the analysis has been doubled. The oil content has been determined by the chemical laboratories of the Swedish Seed Association. On the harvests from 1947—1951, incl., the oil content has been determined by a refractometric method developed by LEITHE (1936) with small modifications. From 1952 and on the gravimetric method developed by TROËNG (TROËNG 1955, LINDBERG, TROËNG and LYTTKENS 1956) has been used. The analyses of individual plants have been made on 2—3 grams of seed, those of progeny plots on 5 grams. The progeny plots have been 2.70 sq. meters without replications.

The statistical treatment of the material has been carried out according to the methods described by BONNIER and TEDIN (1940, 1957). Number of individuals=n, mean=$\bar{x}$, regression of y upon x, coefficient b_{yx}, correlation coefficient r. Partial correlation between two characters (x, y) when the third one (z) is constant=$r_{yx.z.}$ Degrees of significance have been marked: *=$0.05 > p > 0.01$; ** $0.01 > p > 0.001$; *** $p < 0.001$. Asymmetry of the curves has been calculated by the formulae given by SCHNELL (1958, pp. 743—746).

II. SELECTION FOR SEED YIELD AND YIELD COMPONENTS

The seed yield of the individual plant of rape or mustard is determined by the number of pods, seeds per pod and seed weight. Among these components the number of pods has by far the greatest influence upon the yield. This is evident already by an ocular inspection of the material and when correlations between pod number and seed yield have been determined values for the correlation coefficient of about +0.90 are usual (SCHRIMPF 1954, STOLLE 1954). It would, therefore seem to be the simplest way for the breeder to select plants either directly for yield or for pod number, especially since the variation in these two characters is very wide. This variation is, however, mainly environmental. Increase in space and thus also in nourishment immediately results in a larger number of branches, more pods and higher yield per plant. According to SCHRIMPF (1954) only about 1/10th to 1/50th of the "flower potential" of KAUFMANN (1942) is developed into flowers in a normal field stand. Even if the "flower potential" is difficult to estimate there is no doubt whatever that the plant is genetically

TABLE 2. *Correlations between the seed yield of the mother plant and that of the progeny plot.*

Crop	n	$\frac{\bar{x}}{g}$	$\frac{\bar{y}}{g}$	b_{yx}	r	t^2
Winter rape 1	90	16.7	1079	2.05	0.12	1.37
„ „ 3	169	17.8	1013	— 0.31	— 0.012	< 1
Summer rape 2	120	10.8	546	8.90	0.33	14.77***
„ „ 3	64	16.8	492	3.12	0.23	3.54
White mustard 1	52	11.2	782	1.08	0.067	< 1
„ „ 4	76	13.8	680	3.70	0.16	1.87

capable of developing a much larger number of flowers and pods than what is possible in the environment of a normal field. STOLLE (1954) reaches the same conclusion by studying the asymmetry of the distribution and he says (p. 206): "Die Modifikation überdeckt hier offenbar die genetische Konstitution recht eindeutig".

It is a common opinion, at Svalöf (ÅKERMAN, personal communication) as well as elsewhere, that the seed weight of an individual plant is a poor measure of the yield of the line derived from the plant (*e. g.* WEBER and MOORTHY 1952, FIUZAT and ATKINS 1953). The first estimate of the yield of the strain, made on small plots without replicates, is also rather uncertain (TEDIN and ANDERSSON 1943) and it is not surprising that usually there is no significant correlation between the yield of the mother plant and that of the progeny. Nor has it been the purpose to investigate more closely the effect of selection after seed yield of the plant; the coefficients presented in Table 2 have been given for a comparison between the effects of selection after yield and after oil content.

Of the six coefficients of correlation only one reaches a level of significance, the remaining five are complety insignificant. It should be observed that the regression coefficients concern *plot* yield on plant yield. Had it been possible to calculate plant yield on plant yield, the values would only have been about 1/100th of the ones given.

Not even in artificial brown mustard (*B. juncea* Coss.) where the low fertility might have made correlation between seed yield of mother and progeny more probable, a significant correlation has been obtained ($r=0.20$, $t^2=0.75$).

The degree of genotypical fixation of the number of pods has not been studied. Judging from the variability of the character and the strong

correlation between number of pods and yield per plant (STOLLE 1954) it is probable that the importance of the genotype for this character is as small as in regard to yield.

The number of seeds per pod and seed size are much less influenced by environment than the number of pods and yield per plant. This is indicated, among other things by the coefficients of variation found by STOLLE (1954), which were 30.0 and 17.1, respectively for the two former characters and 62.0 and 71.3, respectively for the latter.

The correlation between mother plant and progeny plot in regard to number of seeds per pod has been determined in artificial brown mustard. The coefficient of correlation was $+0.85$ ($n=20$, $t^s=46.86^{***}$). This strong correlation is partly dependent upon the great variability in fertility in artificial brown mustard. Also in normal, fully fertile spring rape there is a significant though weaker correlation ($b=+0.25$, $r=+0.36$, $n=120$, $t^s=17.87^{***}$). In white mustard it has been possible by five years continuous selection in positive and negative directions to increase the seed number by 1.33 ± 0.096 respectively decrease it by 1.51 ± 0.121 (Table 12). It is evident that the seed number per pod is so strongly influenced by the genotype that selection for this character will result in a marked differentiation in the material.

The correlation between mother and progeny in regard to 1000-grain weight has only been determined in a material of spring rape. The correlation was fully significant ($b=+0.22$, $r=+0.41$, $n=120$, $t^s=24.17^{***}$). The material is too limited to permit any definite conclusions about the possibilities to select for seed size, but combined with what is known about the variability of the character it seems probable that such a selection would be effective.

III. SELECTION FOR OIL CONTENT

1. The variability of the oil content

The oil content of the seed is modified especially by the weather conditions during ripening, by nutrition and by the degree of ripening at harvest. The fairly large differences in oil content between different years are mainly caused by different weather conditions. If there is sufficient water in the soil, high temperatures during ripening will result in high oil content (SCHWARZE 1958). Among the nutrients nitrogen has the greatest influence, several investigations have shown that at least large dressings with nitrogen decrease the oil content (BJÖRKLUND and

TABLE 3. *Comparison between the standard deviations in oil content of mother plants and progeny plots.*

Crop	n	Standard deviation of			Difference in % of the value of the mother plants	
		mother plants	progenies	Difference		
Winter rape 3	169	2.04[1]	1.27	0.77	37.7	45.9
„ „ 4	69	2.40[2]	1.26	1.14	47.5	
Summer rape 2	120	2.42	1.15	1.27	52.5	
White mustard 2	77	2.39[3]	1.00	1.39	58.2	60.0
„ „ 3	41	2.73[4]	1.08	1.65	60.4	
„ „ 4	76	2.62	1.01	1.61	61.5	

[1] For all the plant material s=1.89.
[2] „ „ „ „ „ s=1.85.
[3] „ „ „ „ „ s=2.25.
[4] „ „ „ „ „ s=2.41.

WAHLGREN 1955, ANDERSSON, OLSSON and LÖÖF 1956). BJÖRKLUND and WAHLGREN estimate that an increase in dressing of 100 kg calcium nitrate per hectare will result in a decrease in oil content with 0.3 to 0.4 per cent. Oil is largely formed in the last stages of ripening and oil content is strongly influenced by degree of ripening; early harvest will result in a low oil content (ANDERSSON and BJÖRKLUND 1945, RADET 1951, WAGNER 1954). In spite of these modifying influences oil content is decidedly less variable than seed yield and the yield components. STOLLE (1954) found in three populations coefficients of variation from 5.5 to 7.0 and in nine populations here recorded (Table 1) the coefficient varied from 3.6 to 8.4.

The use of the coefficient of variation in comparing the variabilities of different populations surmises a general proportionality between mean and standard deviation. The mean oil contents of rape is about 45 per cent and of white mustard about 30 per cent. A study of the variation in oil content between individual plants of rape and white mustard shows, however, that the standard variation in the latter is at least as high as in the former (Table 3), and there is no difference in standard deviation corresponding to that in mean value. For that reason, in comparing the variabilities in these two species the standard deviation must be considered at least as adequate as the coefficient of variation, and in Table 3 the standard deviations are given for the populations, where oil content is determined in per cent of dry matter.

TABLE 4. *Regression and correlation coefficients between the oil contents of mother plant and progeny in material where the oil content has been determined by refractometric method.*

Crop	n	b	r	t^2
Winter rape 1	108	+ 0.37	+ 0.38	18.29***
„ „ 2	134	+ 0.19	+ 0.36	19.35***
Summer rape 1	89	+ 0.27	+ 0.30	8.53**
White mustard 1	52	+ 0.16	+ 0.44	11.86**

In two of the populations listed in Table 3 all individual plants have been used for progeny plots, in the other four mostly plants with more extreme oil contents have been used and a number of those with intermediate values excluded. For the latter the standard deviation among all mother plants analyzed are given in notes to the table. There can be no question about the main fact, that the variation between progeny plots is considerably less than between plants.

This reduction in variability is partly due to the fact that the value for each progeny plot is in reality a mean of about 100 plants which must very strongly reduce that part of the variability which is due to random modifications between plants in a plot. Another cause of the reduction from mother plant to progeny plot is the cross pollination, which must partly even out the genotypical differences existing between the mother plants. It is interesting to note that the total reduction in variability is about 60 % in white mustard with 100 per cent cross pollination but only 46 % in rape which is self pollinating to about 2/3rds.

2. The correlation between oil contents of mother plant and progeny

The oil content in some materials have been determined by a refractometric method, in others by direct determination of oil in per cent of dry matter. In some cases it has not been possible to recalculate the refractometric values in per cent oil of dry matter and therefore the correlation and regression coefficients have been calculated separately for the two groups of material (Tables 4 and 5), in spite of the fact that there is no real fundamental differences between the two groups of results.

As seen from the tables the regression coefficients are rather low but all the correlations are significant, most of them highly so, and a selec-

TABLE 5. *Regression and correlation coefficients between the oil contents of mother plant (x) and progeny (y) in material where the oil content is determined in per cent of dry matter.*

Crop	n	$\bar{x}$	$\bar{y}$	b	r	t^2
Winter rape 3	169	50.61	44.72	+0.20	+0.32	19.53***
„ „ 4	69	46.80	43.43	+0.24	+0.46	18.16***
Summer rape 2	120	43.36	40.79	+0.21	+0.43	27.14***
Sum and mean	358	47.44	43.16	+0.21 ±0.027		
White mustard 2	77	28.56	28.09	+0.20	+0.48	22.13***
„ „ 3	41	27.33	31.35	+0.24	+0.61	23.60***
„ „ 4	76	32.10	31.49	+0.14	+0.37	11.49**
Sum and mean	194	29.69	30.11	+0.19 ±0.026		

tion after oil content of the plant must be expected to give much better results than a selection after seed yield. The analysis of covariance of the rape material in Table 5 shows that the mean values of the different populations deviate significantly from the mean regression within populations ($v^2=60.84$***), nor do they fit a straight regression line for population means ($v^2=25.42$***). On the other hand there are no significant differences between the regression lines within the different populations and the mean regression "within" ($v^2=5.08$). A corresponding analysis of the material of white mustard gives similar results. Since there are no significant varietal differences in regard to regression within population the mean values for each species have been entered in Table 5. In this material the coefficients are about the same in both species, though it might have been expected that rape should have had a higher value than mustard because of its fairly low percentage of cross pollination.

The linearity of the regression has been determined in one population (no. 2) of summer rape and one (no. 2) of mustard. In these there were no indications that the regression is not linear and it must be considered permissible to treat it as linear at least within the range of variation existing in the present material.

In order further to illustrate the effect of selection Table 6 gives the average oil contents of progenies grouped after the oil content of the mother plant in one case each of winter rape, summer rape and white mustard. Using weighted means for the three lowest and the three

TABLE 6. *The oil content of the progenies from mother plants in different oil content classes.*

	Winter rape 3						
The oil content of the mother plant %	41—43	44—45	46—47	48—49	50—51	52—53	54—55
Number of progenies	1	4	18[1]	17[1]	88	40	1
Mean oil content of the progenies in %	45.1	43.7	43.9	44.3	44.7	45.4	47.1
	Summer rape 2						
The oil content of the mother plant %	36—37	38—39	40—41	42—43	44—45	46—47	48—49
Number of progenies	3	7	18	45	31	14	2
Mean oil content of the progenies in %	39.2	39.8	40.5	40.8	40.8	41.6	42.0
	White mustard 4						
The oil content of the mother plant %	25—26	27—28	29—30	31—32	33—34	35—36	37—38
Number of progenies	3	9	12	17	25	9	1
Mean oil content of the progenies in %	30.7	30.7	31.5	31.4	31.7	32.1	31.0

[1] Selected.

highest classes among the mother plants the differences between "positive" and "negative" selections is found to be 0.99 % in winter rape, 0.91 in summer rape and 0.69 in mustard.

3. Some results of individual selection for oil content

That it is possible by individual selection in old varieties to breed new ones with a substantial increase in oil content has been shown *e. g.* by the breeding of Lembke's rape from an old land variety (BAUMANN 1918) and of Vestal from Lembke's via Regal (MANNER 1957). Table 7 shows the differences in oil content between some lines in the present breeding material at Svalöf and their mother varieties.

In winter rape lines have been obtained with a higher oil content than the mother variety both from Lembke's, Matador and Tenus. In white mustard the selection has been made mainly in material treated with

TABLE 7. *Increase in oil content obtained in the breeding work by individual selection within existing varieties.*

	Number of trials	The superiority of the new strain over the mother variety in per cent oil of dry matter	t-values for differences
Winter rape			
Sv Matador (from Lembke's rape)	310	+0.112±0.064	1.75
Sv 52/118 („ „ „)	5	+1.88 ±0.47	4.00*
Sv 56/1170 („ „ „) [1]	3	+2.00 ±0.21	9.52*(*)
Sv 54/810 („ Matador)	7	+0.96 ±0.19	5.05**
Sv 56/1172 („ „)	3	+1.20 ±0.25	4.80*
Sv 53/188 („ Tenus)	5	+1.14 ±0.21	5.43**
Sv 54/181 („ „)	4	+1.63 ±0.25	6.52**
White mustard			
Sv Primex (from Sv white mustard)	62	+0.75±0.13	5.77***
Sv 44/303 („ „ „ „)	14	+1.68±0.13	12.92***
Sv 52/262 („ „ 44/303) [2]	5	+1.34±0.27	4.96**
Sv 54/281 („ „ „) [2]	13	+1.97±0.20	9.85***
Sv 54/294 („ „ „) [2]	8	+1,33±0.43	3.09*

[1] Compared with Matador.
[2] „ „ Primex.

X-rays and here the development of Primex meant a significant increase in oil content with 0.75 per cent (ANDERSSON and OLSSON 1954). A sister line of Primex, Sv 44/303 from X-ray treated Svalöf's white mustard has on an average for 14 trials had 1.68±0.13 per cent more oil than the mother population and has surpased Primex in the same trials with 1.08±0.20 per cent. Repeated selection of individual plants in 44/303 has resulted, *i. a.*, in Sv 52/262, Sv 54/281 and Sv 54/294 which have from 1.33 to 1.97 per cent more oil than Primex and are thus superior to the unselected mother population with 2—3 per cent.

Crossing different varieties has given the best results when summer and winter types of rape and turnip rape have been crossed; the results will be discussed in the next section. In the work with white mustard crosses between varieties have usually given poor results, but Sv 53/268 from Roumanian white mustard×Primex has in 19 trials had on an average 0.95±0.20 per cent higher oil content than Primex.

IV. CROSSES BETWEEN SUMMER AND WINTER FORMS OF RAPE AND TURNIP RAPE

Within the regions where the winter forms of rape and turnip rape are able to stand the winter they are superior in yield to the summer forms. The following data, giving the average yields for the last ten years at Svalöf of one representative variety of each type may give an idea about the differences:

Variety	Seed yield	Rel. value	Oil content	Yield of oil	
	kg/hectare			kg/hectare	Rel.
Lembke's winter rape	3860	100	45.6	1506	100
Regina summer rape	2607	68	41.7	927	62
Lembke's winter turnip rape	2754	100	43.3	1025	100
Gute + Arlo summer turnip rape	2084	76	42.7	758	74

The superior yield of the winter forms must to a large extent depend upon the longer vegetation period but there are probably also specific genes for high yield. In 1945 a breeding program was started including crosses between winter and summer forms of rape and in 1948 similar crosses within turnip rape were included. The main aim of these crosses was to breed summer types with higher yields but in turnip rape also the segregating winter types have been made the subject of some breeding work.

1. Segregation values

It is easy to cross summer and winter forms. F_1 is a summer form but flowers and ripens very late. The count in F_2 is made difficult by the fact that even the winter form may flower in late summer. This is especially evident in the case of early sown winter turnip rape and for this reason it was impossible in 1953 to ascertain the segregation in one F_2 plot of this species. The deviations from the 3 : 1 ratio which are found in the turnip rape material in Table 8 are most probably caused by the difficulty in distinguishing some winter types from the late heterozygote.

In the cross between Weihenstephan summer rape and Lembke's winter rape studied by TROLL (1947) as well as in the material presented in Table 8 one recessive factor causes the inhibition of shooting in spring sown winter rape. By crossing a large number of spring- and winter

TABLE 8. *Segregation in F_2 in the summer-winter character in crosses between summer and winter forms of rape and turnip rape.*

Cross combination	F_2 year and number	Segregation				P values
		Absolute values			Frequencies	
		Summer forms	Winter forms	Total		
Rape						
Sv Regina summer rape × Lembke's winter rape	1947—371	2091	688	2779	3.01 : 0.99	$0.7 < P < 0.8$
„ „ „ „ × Sv Late winter rape	1949—361	983	337	1320	2.98 : 1.02	$0.6 < P < 0.7$
„ „ „ „ × Sv Tenus winter rape	1949—362	1608	512	2120	3.03 : 0.97	$0.3 < P < 0.4$
Total		4682	1537	6219	3.01 : 0.99	$0.6 < P < 0.7$
Turnip rape						
Sv 0300 × Sv 45/140 from Ukraine winter turnip rape	1950—362	954	252	1206	3.16 : 0.84	$P < 0.001$
„ „ × Lembke's winter turnip rape	1951—363	359	105	464	3.09 : 0.91	$0.2 < P < 0.3$
„ „ × Sv Rapido winter turnip rape	1952—362	660	182	842	3.14 : 0.86	$0.02 < P < 0.05$
„ Arlo × Sv 45/140 from Ukraine winter turnip rape	1952—363	220	68	288	3.06 : 0.94	$0.5 < P < 0.6$.
Total		2193	607	2800	3.13 : 0.87	$P < 0.001$

Sv 0300 from Mette's summer turnip rape.

wheat varieties it has been possible to demonstrate the existence of mono-, di- and trigenic segregations for this character in *Triticum vulgare* (ÅKERMAN 1943, ÅKERMAN and MAC KEY 1949) and it is possible that further study will show the existence of more than one factor pair especially in the amphidiploid rape.

The rhythm of development of the plants is not influenced solely by the summer-winter gene and among homozygous summer types there is still a considerably segregation in regard to earliness. The cross between the two widely different types, summer and winter, results in complicated segregations, caused by polymeric genes, in such characters as rhythm of development, ripening time and tendency for forming green shoots. The first segregation in F_2 must be repeated several generations in order to obtain evenly ripening varieties. The best strains now tested from the cross Regina summer rape×Lembke's winter rape ripen 2—5 days later than Regina and families from the cross Sv 0300 (from Mette's) summer turnip rape×Lembke's winter turnip rape are 3—6 days later than Arlo summer turnip rape, which in its turn is later than Sv 0300. By backcrossing with pure summer forms it is possible to obtain earlier types.

2. The practical value of the crosses

Lembke's winter rape is superior to Svalöf Late rape and Tenus in yield and among the combinations with Regina summer rape the ones with Lembke's winter have given the most promising results. There is a general tendency for crosses with winter rape to increase the oil content of the summer type. In order to illustrate this fact Table 9 presents the oil contents in 1957 and 1958 of all analysed progeny plots of spring rape from certain crosses.

The oil content is highest in the cross Regina×Lembke's and the superiority over Regina is on an average 2.43 ± 0.23 per cent oil of dry matter. Backcrossing with Regina or with Janetzki's summer rape causes a decrease in oil content but this is still significantly higher than in Regina. In 1958 Regina is compared on the one hand with its cross with Janetzki's on the other with the cross between the latter and Sv 52/215 which latter is derived from Regina×Lembke's winter rape. The differences to Regina are 0.46 ± 0.25 per cent ($t=1.84$) and 1.27 ± 0.21 percent ($t=6.05^{***}$), respectively.

Oil content and oil yield from the best strains from the cross Regina

TABLE 9. *Oil content in progeny plots in 1957 and 1958.*

Variety or combination	Oil content in per cent of dry matter									n	$\bar{x}$
	38	39	40	41	42	43	44	45	46		
1957											
Regina summer rape			2	9	7	2				20	41.95±0.18
Regina × Lembke's winter rape				2	3	19	22	13	5	64	44.38±0.14
(Regina × Lembke) × Regina			2	4	9	14	5	3	1	38	43.26±0.22
(Regina × Lembke) × Janetzki				4	12	10	12	2		40	43.40±0.17
1958											
Regina summer rape		6	7	2						15	40.23±0.18
Sv 52/215 × Janetzki		4	16	36	14	5				75	41.50±0.11
Regina × Janetzki	1	9	13	8	5					36	40.69±0.18

summer rape×Lembke's winter rape are presented in Table 10. The superiority in oil content over Regina is evident. The superiority in oil yield is more than 10 per cent but in this case the significance is weaker than in regard to oil content. The reason is that these varieties have been tested also in Central Sweden where they have given poor results in years with a late harvest.

Winter rape has larger seeds than summer rape and the seeds of the hybrid strains are often larger than these of the summer form (Table 10). Sv 52/215, Sv 53/93 and Vg 53/8 outyield Regina in seed with 4—7 per cent and at the same time their seeds are 5—8 per cent heavier than those of Regina. In this case the superiority in yield seems to depend largely upon an increase in seed size. It is of interest to note that the derivatives of the crosses between winter and summer rape are superior to the latter parent both in seed size and oil content. Within the strain 55/102, derived from a similar cross the two characters show negative direct correlation ($t^2=7.05^{**}$, summer rape 4 in Table 26).

Among the varieties of winter turnip rape used in the crosses the variety Lembke's has a higher yield than Rapido and Sv 45/140 from an Ukrainian winter turnip rape. As summer component Sv 0300 has been mainly used, a strain derived from the early German variety Mette's summer turnip rape. Sv 0300, however, has a fairly low yield and both in large scale cultures and as standard variety in the trials it has been superseded first by Gute and then by Arlo. The oil content of

TABLE 10. *Oil content and oil yield of the best strains tested from the cross Regina summer rape × Lembke's winter rape in comparison with Regina summer rape.*

Strain	Number of trials	Oil in % of dry matter			Oil yield, kg/hectare			1000 grain weight			
		Regina summer rape	Strain minus Regina	t	Regina summer rape	Strain minus Regina	t	n	Regina summer rape	Strain minus Regina	t
Sv 52/215	17	39.52	+ 2.06 ± 0.24	8.58***	555	+ 55 ± 19.5	2.82*	13	3.56	+ 0.188 ± 0.054	3.48**
Vg 53/8	17	40.50	+ 1.77 ± 0.40	4.43***	495	+ 52 ± 18.3	2.84*	14	3.56	+ 0.257 ± 0.058	4.43***
Sv 53/93	15	39.49	+ 1.99 ± 0.32	6.22***	556	+ 63 ± 16.9	3.73**	10	3.61	+ 0.281 ± 0.082	3.42**
„ 53/95	4	41.10	+ 2.05 ± 0.30	6.83**	956	+ 126 ± 25.3	4.98*	2	3.81	+ 0.26	—
„ 56/180	3	43.27	+ 2.13 ± 0.69	3.09	1186	+ 144 ± 50.5	2.85	2	3.43	+ 0.08	—

TABLE 11. *Oil yield of some strains from crosses between winter and summer turnip rape in comparison with that of Arlo summer turnip rape.*

Combination	Relative yield of oil Arlo = 100								Number of strains
	90	95	100	105	110	115	120	125	
From Sv 0300×Sv 45/140 from Ukraine		1	9	5	1	1			17
From Sv 0300×Lembke's turnip rape				2	4	7	8	1	22

Arlo is about 0.3 per cent units higher than that of Sv 0300 while the superiority in oil yield is about 7 per cent (ANDERSSON 1952, 1958).

Most of the work has been done with the combinations Sv 0300×Sv 45/140 and Sv 0300×Lembke's, the latter has given the most promising results. The different strains from the crosses summer×winter turnip rape have only been compared with Arlo in three years trials. For this reason Table 11 gives a summary of the oil yields of all families tested instead of more detailed data for some of the families.

Nearly all of the strains from Sv 0300×Lembke's have surpassed Arlo with 10—25 per cent in the trials at Svalöf. The increase in oil yield is mainly caused by an increase in seed yield and this again is to large extent dependent upon larger seeds. The increase in oil content — in comparison with "normal" summer forms — is less in the summer× winter combinations of turnip rape than in the corresponding crosses in rape; the reason is probably that the differences between the parents is less in the former species than in the latter. On an average, however, all the tested strains from the combination Sv 0300×Lembke's have 0.87 ± 0.090 ($t=9.77^{***}$) higher oil content than Arlo.

Crosses between summer and winter forms of rape and turnip rape make it possible to select new varieties of the summer type which are much superior to the old ones in regard to oil content and oil yield. The possibility to utilize the crosses even in the breeding of winter types has not been thoroughly studied — mainly, perhaps, because the use of artificial types seemed more promising. In some of the turnip rape crosses continuous selection for winter types has been undertaken since 1952, however. At first the material had a very poor winter hardiness but this has gradually improved. By a proper selection of parents it seems possible to obtain transgressions in oil content without losses in hardiness.

V. CONTINUOUS SELECTION FOR SEED NUMBER PER POD AND OIL CONTENT IN WHITE MUSTARD

Very striking examples of the possibility to change a cross fertilizing population by continuous selection are shown in maize, where HOPKINS in 1896 started selection for high and low oil content and for high and low protein content; the work has been continued for 50 generations and resulted in a very strong differentiation in the material. The oil content was 4.70 per cent in the original material, it has been increased on the one hand to 15.36 per cent and decreased on the other to 1.01 per cent. The corresponding results in regard to protein are from the initial 10.92 per cent to 19.54 and 4.91 per cent, respectively (WOODWORTH, LENG and JUGENHEIMER 1952). In breeding *Taraxacum koksaghys* at Svalöf it was possible already after 5 generations to double the rubber content (JOSEFSSON 1953). In view of these experiences an attempt was started in 1952 to differentiate a population of white mustard in regard to oil content and number of seeds per pod.

1. Methods

As starting material was chosen the Svalöf variety Primex white mustard, selected from X-ray treated material and the only variety grown in Sweden. The first plot for selection comprised about 5000 plants. In the field a few hundred plants were selected which showed poor pods with few seeds. These were threshed, seeds per pod counted and the 50 plants with the lowest values were chosen as the minus selection for seeds per pod. Also in the field a selection was made for well developed plants with "good" pods. 398 of these plants were also threshed and the number of seeds per pod determined; in 321 of these cases also the oil content was determined. Among the 398 plants the 50 ones with the highest seed number per pod were chosen for the positive selection for this characters; plus and minus selections for oil content were both taken from the 321 plants analyzed.

The four sub-populations thus formed were sown next year on the one hand in distance-isolated plots for further selection, on the other together with unselected material in a small trial with four replicates. In the latter material 100 plants, divided between the replicates, were chosen at random and used for determination of seed yeild, number of seeds per pod, 1000-grain weight and oil content. The plots for further selection have included some 2.500 plants for the positive series and some 2.000 for the negative ones. In the plots for selection after number of

seeds per pod a preliminary selection has been made in the field, a few hundred obvious plus- respectively minus-variants have been harvested and the number of seeds then determined for 20 pods on each plant, the extremes in each case being selected for next years population. During 1955 and 1956 the plants in the plus series have been selected to some extent also for high oil content. In the plots for selection after oil content a selection in the field has been made for well developed plants with long pods and a high number of "seed-knots" on the pods. In the 1955 and 1956 selections for high oil content there was also a certain amount of direct selection for high number of seeds per pod. About 10 % of the plants in the plots for selection after oil content have been analyzed and about 20 % of the plants analyzed have been used for the progeny populations which in all cases have been built upon about 50 individual plants. The selection in the field for general vigour and the partial combination of selection for high oil content with a selection for many seeds per pods and *vice versa* is caused by the fact that the scheme has been carried out not solely as a methodical study but also as a practical breeding program in an attempt to breed varieties which combine high yield with high oil content. In judging the relations between the characters due consideration must be given the partial coupling of the two in some cases.

Fifty plants have been used to build each new selected population in order to avoid any risks for inbreeding depression and in view of the fact that if further selection is desired it is easy to limit the genetical variation but not to widen it. Tests of individual plant progenies before the combination into progeny populations would probably have given a stronger effect of selection but would have taken longer time and been more expensive.

TABLE 12. *The direct effect upon number of seeds per pod of continuous selection for this character in white mustard (n=100).*

Year	Generation	Selection for high seed number		Unselected. Number of seeds per pod	Selection for low seed number	
		Diff.	t		Diff.	t
1953	1	$+0.16\pm0.113$	1.42	5.76	-0.55 ± 0.128	4.30^{***}
1954	2	$+0.52\pm0.101$	5.15^{***}	5.74	-0.66 ± 0.096	6.88^{***}
1955	3	$+0.84\pm0.095$	8.84^{***}	5.17	-0.70 ± 0.088	7.95^{***}
1956	4	$+1.15\pm0.100$	11.50^{***}	5.74	-1.00 ± 0.109	9.17^{***}
1957	5	$+1.33\pm0.096$	13.85^{***}	5.87	-1.51 ± 0.121	12.48^{***}

2. The effect of selection after number of seeds per pod

The direct effects of selection after number of seeds per pod are seen in Table 12. After five years the number of seeds is 7.20 in the positive selection, 5.87 in the standard and 4.36 in the negative selection; the differences are highly significant.

As to indirect effects of the selection for seed number there is a tendency towards smaller seeds with large number and larger seeds with small number (Table 13). This is also evident from the fact that there is nearly always a negative correlation between seed number and seed size (Table 14). The attempts to increase the yield per pod by an increase in the number of seeds is thus to some extent counteracted by a simultaneous decrease in seed size. This latter, however, only partly neutralizes the effect of the increase in number. In 1957, when the difference in seed size reached its maximum the average weight of seeds per pod was 56.7 mg in "High number", 47.4 mg in unselected and 39.3 mg in "Low number".

The total yield of a plant is, as has been pointed out, a poor measure of its genotypical productivity. It is impossible to achieve a fully even stand and the yield per plant is mainly determined by the number of pods; only in 1957 the higher number of seeds per pod has also resulted in a significantly higher yield (Table 13). The direct correlation between yield per plant and number of seeds per pods is significant only in 5 cases out of 15; the partial correlation, however, with 1000-grain weight constant reaches in 12 cases of 15 P-values below 0.05 (Table 14).

A strongly significant positive correlation is found between seed size and yield per plant, both as direct correlation and as partial with seed number kept constant. Probably this is at least partly due to parallel modifications of pod number and seed size, which are both positively influenced by an increase in available nutrients.

In the population with high number of seeds the oil content is somewhat higher than in the unselected material in 1956 and 1957 but this is most probably due to the selection also for oil content in this material. The correlation between seed number and oil content is usually positive, but if the influence of seed size is eliminated the correlation reaches a P-level of less than 0.05 only in 2 cases out 15 (Table 14). There is no strong relation between seed number per pod and oil content; anyway there is no indication whatever that an increase in seed number should make it more difficult to increase the oil content.

TABLE 13. *The indirect effect of selection for number of seeds per pod, upon seed yield, 1000-grain weight and oil content of individual plants in white mustard (n=100).*

Year	Generation	Seed yield of the plant (g.)					1000-grain weight (g.)					Oil content per cent of dry matter				
		Selection for high seed number		Unselected	Selection for low seed number		Selection for high seed number		Unselected	Selection for low seed number		Selection for high seed number		Unselected	Selection for low seed number	
		Diff.	t		Diff.	t	Diff.	t		Diff.	t	Diff.	t		Diff.	t
1953	1	−1.28±0.75	1.71	10.85	−1.43±0.75	1.91	—	—	—	—	—	+0.03±0.35	0.09	32.49	+0.10±0.35	0.29
1954	2	+0.69±0.83	0.83	9.76	−0.38±0.79	0.48	−0.01±0.15	0.07	7.30	+0.18±0.15	1.20	−0.49±0.37	1.32	28.89	−0.28±0.39	0.72
1955	3	−0.48±0.82	0.59	9.63	+0.88±0.85	1.04	+0.07±0.18	0.39	8.59	+1.05±0.19	5.53***	−0.07±0.28	0.25	27.75	−1.14±0.33	3.45***
1956	4	+1.51±1.23	1.23	12.56	+2.48±1.31	1.89	−0.14±0.17	0.82	8.65	+0.17±0.19	0.89	+0.83±0.36	2.31*	30.30	+0.17±0.38	0.45
1957	5	+3.67±1.35	2.72**	17.18	−0.50±1.16	0.43	−0.21±0.14	1.50	8.08	+0.94±0.21	4.48***	+0.65±0.33	1.97(*)	30.87	−0.65±0.36	1.81

TABLE 14. *Summary of the correlations between different characters in the white mustard material in 1955—57.*

Characters correlated		Number of correlations			Number of correlations with P-values			
		positive	negative	total	>0.05	$0.05-0.01$	$0.01-0.001$	<0.001
Number of seeds per pod (x) and 1000-grain weight (z)	r_{zx}	1	14	15	7	3	1	4
Number of seeds per pod (x) and yield of seed per plant (v)	r_{vx}	14	1	15	10	4	1	—
Number of seeds per pod (x) and yield of seed per plant (v)	$r_{vx \cdot z}$	15	0	15	3	6	3	3
1000-grain weight (z) and yield of seed per plant (v)	r_{vz}	15	0	15	2	—	7	6
1000-grain weight (z) and yield of seed per plant (v)	$r_{vz \cdot x}$	15	0	15	—	1	2	12
Number of seeds per pod (x) and oil content (y)	r_{yx}	13	2	15	10	2	1	2
Number of seeds per pod (x) and oil content (y)	$r_{yx \cdot z}$	13	2	15	13	2	—	—
1000-grain weight (z) and oil content (y)	r_{yz}	0	15	15	1	2	2	10
1000-grain weight (z) and oil content (y)	$r_{yz \cdot x}$	0	15	15	1	2	4	8
Yield of seed per plant (v) and oil content (y)	r_{yv}	2	13	15	10	1	2	2
Yield of seed per plant (v) and oil content (y)	$r_{yv \cdot zx}$	8	7	15	14	1	—	—

TABLE 15. *The direct effect upon oil content of continuous selection for this character in white mustard (n=100).*

Year	Generation	Selection for high oil content		Unselected. Oil in % of dry matter	Selection for low oil content	
		Diff.	t		Diff.	t
1953	1	+0.83±0.367	2.26*	32.49	−0.47±0.356	1.32
1954	2	+0.76±0.362	2.10*	28.89	−1.35±0.390	3.46***
1955	3	+0.63±0.303	2.08*	27.75	−2.31±0.294	7.86***
1956	4	+2.60±0.360	7.22***	30.30	−2.69±0.371	7.25***
1957	5	+3.07±0.323	9.50***	30.87	−2.45±0.327	7.49***

3. The effect of selection after oil content

The direct effect of selection after oil content is shown in Table 15. After five years selection the oil content in the plus series is 33.94 %, in the standard 30.87 % and in the minus series 28.42 %; the differences are fully significant. Among other changes the increase in seed number per pod is most marked (Table 16). Already the selection by eye in the field after pod development and number of "seed-knots", which has been made all years in the low oil content series and until 1955 in the high content series, has resulted in a significant increase in seed number and this increase becomes still more marked when selection has been made directly for number of seeds in the high oil content series progeny tested in 1956—1957. This result further supports the opinion that oil content can be increased independently of an increase in seed number per pod.

In three cases the high oil content series has had a higher plant yield than the standard, in two cases a lower one; the low oil content series has yielded more seed per plant than standard in two cases, less in three. The correlation between seed yield and oil content has been negative in all cases which is to be expected from the negative influence of nitrogen upon oil content already mentioned. The correlation is weak, however, and only in 5 cases out of 15 does the direct correlation reach any level of significance. The partial correlation, with number of seeds and 1000-grain weight constant reaches the "one star" level only in one case.

The selection for oil content has not resulted in any clear differentiation of the populations in regard to seed size, but there is a tendency towards increased size in "low oil content" in 1957. The correlation between oil content and seed size is negative in all cases, however, significantly so in 14 cases of 15 (Table 17). There is a risk for a

TABLE 16. *The indirect effects of continuous selection for oil content upon seed yield, number of seeds per pod and 1000-grain weight of individual plants in white mustard (n=100).*

Year	Generation	Seed yield of the plant (g.)					Number of seeds per pod					1000-grain weight (g.)				
		Selection for high oil content		Unselected	Selection for low oil content		Selection for high oil content		Unselected	Selection for low oil content		Selection for high oil content		Unselected	Selection for low oil content	
		Diff.	t		Diff.	t	Diff.	t		Diff.	t	Diff.	t		Diff.	t
1953	1	−1.50±0.75	2.00*	10.85	−1.61±0.72	2.24*	−0.09±0.120	0.75	5.76	−0.16±0.119	1.34	—	—	—	—	—
1954	2	+1.38±0.86	1.60	9.76	−0.90±0.76	1.18	+0.15±0.108	1.39	5.74	+0.02±0.101	0.20	—	—	—	—	—
1955	3	−1.97±0.82	2.40*	9.63	−2.07±0.74	2.80**	+0.28±0.100	2.80**	5.17	+0.18±0.091	1.98	+0.21±0.17	1.24	8.59	+0.06±0.17	0.35
1956	4	+3.71±1.30	2.85**	12.56	+1.23±1.26	0.98	+0.84±0.101	8.32***	5.74	+0.31±0.108	2.87**	−0.16±0.18	0.89	8.65	+0.10±0.18	0.56
1957	5	+2.88±1.32	2.18*	17.18	+0.68±1.21	0.56	+0.81±0.104	7.79***	5.87	+0.48±0.105	4.57***	−0.03±0.15	0.20	8.08	+0.32±0.15	2.13*

TABLE 17. *Correlations between 1000-grain weight (z) and oil content (y) in white mustard 1955—1957 (n=100).*

Variety	1955				1956				1957			
	b_{yz}	r_{yz}	$r_{yz \cdot x}$	t^2 for $r_{yz \cdot x}$	b_{yz}	r_{yz}	$r_{yz \cdot x}$	t^2 for $r_{yz \cdot x}$	b_{yz}	r_{yz}	$r_{yz \cdot x}$	t^2 for $r_{yz \cdot x}$
High oil content	−0.51	−0.30	−0.28	8.14**	−0.45	−0.23	−0.22	4.95*	−1.05	−0.48	−0.48	28.84***
High seed number	−0.49	−0.35	−0.33	11.57***	−0.29	−0.13	−0.084	0.69	−1.36	−0.55	−0.54	39.68***
Unselected	−0.92	−0.51	−0.50	32.33***	−0.73	−0.36	−0.29	8.77**	−1.11	−0.53	−0.49	30.86***
Low oil content	−0.42	−0.25	−0.24	6.17*	−0.67	−0.32	−0.30	9.31**	−1.16	−0.51	−0.52	35.14***
Low seed number	−0.55	−0.33	−0.32	11.15**	−0.92	−0.46	−0.35	13.83***	−0.99	−0.60	−0.45	25.23***

The direct correlations reach the P-value of 0.05 if r is at least 0.20.
The direct correlations reach the P-value of 0.01 if r is at least 0.26.
The direct correlations reach the P-value of 0.001 if r is at least 0.32.

TABLE 18. *Comparison between Primex, Sv 54/122 and Sv 54/123 in trials at Svalöf 1954—1958.*

	Number of trials	Primex	Sv 54/122	Sv 54/123
Seed yield, kg/hectare	5	2864	3009	2867
„ „ , relative values	5	100	105	100
Oil in % of dry matter	5	29.54	31.82	30.30
„ yield, kg/hectare	5	719	812	737
„ „ , relative values	5	100	113	103

decrease in oil content if a one-sided selection for large seeds is made within a variety or a cross population. In comparing different varieties and species, however, there is at least in many cases no negative correlation between seed size and oil content.

4. The practical value of the positive selections

In order to estimate the practical value of the positive selections these have been tested in the variety trials at Svalöf during 1954—1958. The selection for oil content has been designated as Sv 54/122 and that for high number of seeds per pod as Sv 54/123. The results are presented in Table 18. In these trials Sv 54/122 has outyielded Primex with 5 % in regard to seed and with 13 % in regard to oil. The difference in oil content has been 2.28 per cent units. Sv 54/123 on the other hand has the same seed yield as Primex in spite of the selection for high number of seeds per pod. The result is in agreement with the weak correlation between number of seeds per pod and plant yield already mentioned (Table 13). An increase only in seed number per pod does not warrant a higher yield.

Sv 54/122 has been tried in comparison with Primex also at some of the branch stations of the Swedish Seed Association and also in some local variety trials. In all the seed yield of the varieties have been compared in 31 trials and in 27 of these the oil content has been determined. The results are summarized in Table 19.

In these trials the oil content of Primex has been 30.09 % while Sv 54/122 has had 31.83 %; the difference 1.74 % is fully significant. The increase in oil content has been achieved without any decrease in seed yield and the result is that Sv 54/122 outyields Primex with about 8 % in oil. It should be observed that this figure does not represent the result of five years selection, since selection and testing are running

TABLE 19. *Seed yield, oil content and oil yield of Sv 54/122 in comparison with Primex in all trials 1954—1958.*

	Number of trials	Primex	Sv 54/122	Diff.	t
Seed yield, kg/hectare	31	1612	1664	52±76.8	0.68
Oil in % of dry matter	27	30.09	31.83	1.74±0.175	9.94***
„ yield, kg/hectare	27	419	454	35±9.4	3.72***

parallely; when the trials started in 1954 the selection had only been carried out for 2 years.

5. The effects of selection upon the variability of the characters

WINTER (1929) has made a thorough study of the effects of 28 years of continuous selection in maize for high and low oil content and high and low protein content on the variability of the characters in question. If the coefficient of variation is used as a measure of the variability, this has decreased in the positive but increased in the negative series. If the variability is measured by standard deviation, by WEINBERG's formula or by the extramodal coefficient it is found to increase when the segregation results in a higher mean value and *vice versa*. The long years of selection must have resulted in a decrease in genotypical variation and the increase in total variation is considered to be caused by the fact that "there is more material present for the environment to interact with".

The present material of white mustard has been selected only for five years and it is too early to make a similar thorough analysis of variability. In Fig. 1, however, the standard deviations are presented in the characters after which selection has been made. Neither in regard to number of seeds nor to oil content is there any indication that the selection so far has changed the variability in the population.

In order to see whether the selection has caused an increase in the relative numbers of extreme individuals the asymmetry of the 1957 material has been determined with the method of SCHNELL (1958). For high and low seed numbers, asymmetry coefficients of +0.071 and −0.029 respectively were obtained and for high and low oil contents −0.230 and −0.140, respectively. With $n=100$ the standard error of the asymmetry coefficient is ±0.2414 and the selection has so far not resulted in any evident asymmetry.

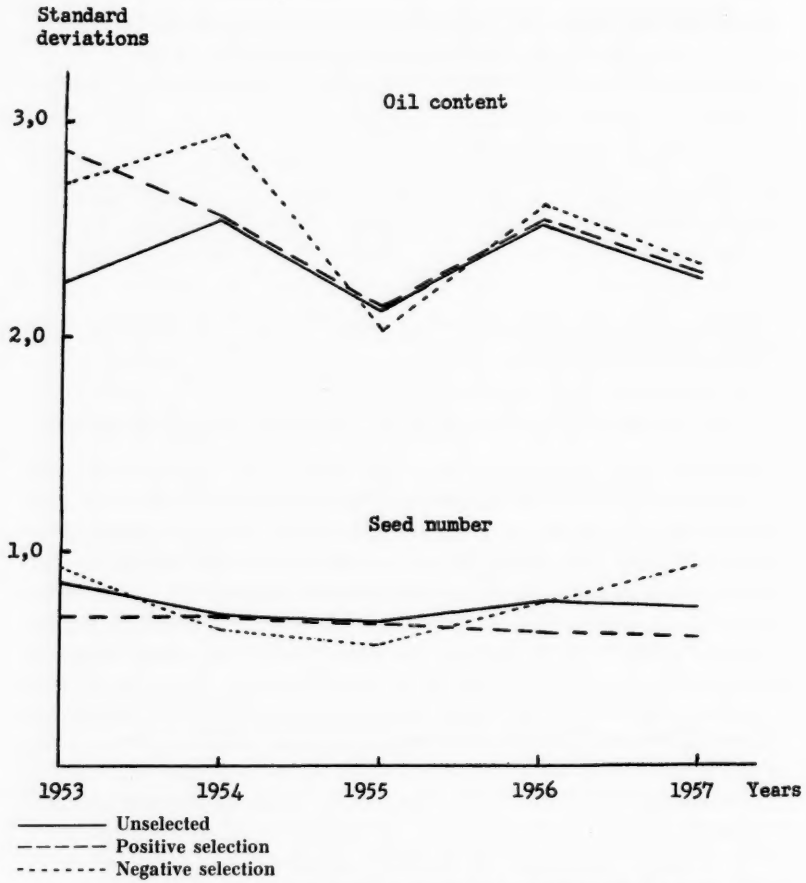

Fig. 1. The effects of continuous selection on the standard deviations in oil content and number of seeds per pod.

6. The influence of selection after number of seeds per pod upon pollen fertility

According to MÜNTZING (1939) intraspecific sterility is a very usual phenomenon especially in diploid cross-fertilizing species. If this holds true also in regard to white mustard the change in seed number per pod may possibly be entirely due to a change in fertility. It is also possible that the number of ovules per pod has increased or that both number of

TABLE 20. *Pollen fertility in the different white mustard populations.*

Variety	Year	The percentage of well developed pollen grains																	n	$\bar{x}$ [1]
		15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	
High oil content	1953											1	—	—	1	1	1	3	73	80	97.6
High seed number	1953															1	1	7	71	80	98.0
Unselected	1953																1	1	78	80	98.4
Low oil content	1953																	2	78	80	98.7
Low seed number	1953										1	—	—	—	—	1	2	1	75	80	97.7
High seed number	1957													1	1	1	4	15	78	100	95.8
Unselected	1957												1	1	—	1	5	22	70	100	95.1
Low seed number	1957			1	2	—	1	1	—	3	1	5	—	1	1	2	5	19	58	100	88.7

[1] The mean values are calculated on the absolute values and not on the classified values.

ovules and fertility have changed simultaneously. The fertility of the material has been studied after one year and after five years of selection. It is difficult to determine the seed fertility, and fertility has thus been determined only as the precentage of well developed pollen grains. The results are presented in Table 20. This table shows that the degree of partial sterility in white mustard is lower than the one shown by MÜNTZING (1939) to exist *e. g.* in rye. If, according to MÜNTZING plants with more than 10 % bad pollen are counted as partially sterile the frequency of such plants is ca 3 % in 1953 and 7—8 % in 1957 except in series " low seed number".

Already the original population is fully pollen-fertile and there is probably a much lower frequency of chromosomal disturbancies in the present population of white mustard than in the rye population studied by MÜNTZING. In my own studies of the fertility in artificial material of rape and brown mustard, the seed fertility has always been somewhat lower than the pollen fertility and it seems probable that the same condition prevails in white mustard. On the other hand there is a strong correlation between pollen and seed fertility and it is probable that a material with such a very high pollen fertility as the present material of white mustard has also a fairly high seed fertility. The increase in number of seeds per pod in 1957 was as much as 22.4 % and it is very unlikely that all of this increase should depend upon an increase in seed fertility. It is instead most probable that the main part of the increase in seed number is caused by a direct increase in the number of ovules per pod. Also the extremely high numbers of seeds obtained by HOPFE (1958) in some cases indicate an increase in number of ovules per pod.

The selection for low number of seeds per pod has also resulted in a poorer fertility and the difference in pollen fertility between unselected and "low seed number" was in 1957 6.4 ± 1.80 %, $t = 3.56^{***}$. The use of the standard error is somewhat doubtful with these extremely asymmetric distributions. If the material is grouped in four fertility classes (< 65, 65—90, 90—95, 95—100) a comparison between low number and unselected gives $\chi^2 = 15.40$ which is very nearly the value required for $P < 0.001$. There can thus hardly be any doubt that the selection for low seed number has resulted in an accumulation of chromosomal aberrants in the material. Probably, however, the whole decrease in number of seeds per pod, 25.7 % in 1957 is not due to this phenomenon alone but also to a definite decrease in the number of ovules per pod.

TABLE 21. *The immediate influence of the father upon the oil content of the seed.*

Combination		Number of plants	Oil content Mean value
Mother	Father		
1 High oil content	High oil content	80	29.24
2 Low oil content	High oil content	80	23.64
3 High oil content	Low oil content	80	30.24
4 Low oil content	Low oil content	80	23.31

VI. THE IMMEDIATE INFLUENCE OF THE FATHER UPON THE OIL CONTENT OF THE SEED

The white mustard seed is nearly completely filled by the embryo, the endosperm on the whole forming a single layer of cells under the seed coat. The oil, thus, is stored in the cotyledons of the following generation. It is considered of interest to investigate whether the oil content is influenced directly by the father, or whether it is wholly determined by the assimilation of the mother plant. An attempt was made in 1958 to determine the influence of the father plant. In a plot with high oil content some plants with low oil content were included and *vice versa*. Since white mustard is 100 % cross pollinated it was possible to obtain from these plots plants representing the four combinations mentioned in Table 21. Four plots have been available for each combination and 20 plants from each plot have been used for analysis of the oil content. The mean values for the different combinations are summarized in Table 21 and an analysis of variance of the material is given in Table 22. As seen from the table the genotype of the mother has a strong influence upon the oil content of the seed while the father does not have any influence whatever. The oil content is determined by

TABLE 22. *Analysis of variance of the data in Table 21.*

Cause of var.	DF.	Sum of squares	Variance	Quotients
Between mother varieties	1	3138.14	3138.14	763.54***
Between father varieties	1	8.62	8.62	2.10
Between replications	3	111.23	37.08	9.02**
Within varieties	314	1291.51	4.11	
Total	319	4549.50		

the genotype of the assimilating mother, not by that of the storing seed. In spite of the fact that the father plant does not influence the oil content as such it is still possible that it may influence the quality of the oil or the enzymatic activities in the seed. MANGELSDORF and FRAPS (1931) have shown that in maize there is a direct relation between the content of vitamin A and the number of genes for yellow pigment in the endosperm.

VII. THE INFLUENCE OF AUTOPOLYPLOIDY UPON THE OIL CONTENT

Autotetraploids have been produced from rape, turnip rape, black mustard (*Brassica nigra* KOCH), brown mustard and white mustard. The tetraploids of turnip rape, black mustard and white mustard are vegetatively well developed while those of rape and brown mustard are vegetatively considerably weaker than the corresponding diploids. Diploids and tetraploids grown side by side easily intercross with aborting or very small triploid embryos as the result (OLSSON and RUFELT 1948). It is, therefore, difficult to compare the yields of diploids and tetraploids. It is easier to compare the size of seeds and their chemical composition since in these cases the abortive triploids are of less importance. In planning the trials it has also been attempted to reduce the risks for inter-ploid crosses to a minimum. The 1000-grain weight, oil content and protein content of some diploid and tetraploid forms of winter turnip rape, summer turnip rape, black mustard and white mustard are presented in Table 23. The tetraploid is compared with the corresponding diploid in all cases except the tetraploid of white mustard from Lembke, where diploid Primex has been used for comparison. The tetraploids tested have been grown for some years but on the whole they are to be considered as raw tetraploids which consistent breeding may considerably improve. From the values in the table it is evident, however, that the increase in seed size of the tetraploids is combined with a considerable decrease in oil content and nearly as large an increase in protein. ARMSTRONG (1950) reports an oil content of 23.5—34.2 % in different tetraploid strains of turnip rape when the corresponding diploid had 38.0 %. SCHWANITZ (1949) reports a decrease in oil content in tetraploid white mustard and oil radish (*Raphanus sativus* L. var. *oleiferous* METZG.) but a somewhat higher oil content in tetraploid turnip rape.

The influence of the cell size upon the metabolism of the plant has

TABLE 23. *The 1000-grain weight, oil content and protein content of some diploid and tetraploid forms of turnip rape, black mustard and white mustard.*

Species and variety	1000-grain weight				Oil in % of dry matter				Protein in % of dry matter			
	n	2x	4x	Diff. 4x–2x	n	2x	4x	Diff. 4x–2x	n	2x	4x	Diff. 4x–2x
Winter turnip rape:												
Lembke type	3	3.49	4.69	+ 1.20	4	46.0	41.4	– 4.6	2	24.9	27.6	+ 2.7
Wilnensis type	3	2.37	3.43	+ 1.06	4	44.9	42.7	– 2.2	2	24.8	28.1	+ 3.3
Summer turnip rape	3	2.61	3.98	+ 1.37	4	42.9	38.5	– 4.4	2	26.5	29.5	+ 3.0
Black mustard	3	1.70	2.79	+ 1.09	6	33.3	29.4	– 3.9	2	30.4	33.4	+ 3.0
White mustard:												
Tetraploid from Svalöf	2	7.93	13.63	+ 5.70	4	29.6	24.7	– 4.9	2	32.2	36.6	+ 4.4
Sv 44/610	3	6.53	10.65	+ 4.12	3	30.0	24.7	– 5.3	3	31.7	35.4	+ 3.7
Sv 44/613	3	6.53	11.18	+ 4.65	4	30.0	25.3	– 4.7	4	31.7	35.9	+ 4.2
Sv 44/617	3	6.53	10.27	+ 3.74	4	30.0	25.8	– 4.2	4	31.7	35.4	+ 3.7
Sv 44/618	3	6.53	10.30	+ 3.77	4	30.0	24.7	– 5.3	4	31.7	35.8	+ 4.1
Sv 44/656	3	6.53	10.61	+ 4.08	3	30.0	24.8	– 5.2	3	31.7	36.6	+ 4.9
Sv 44/657	3	6.53	10.45	+ 3.92	4	30.0	25.9	– 4.1	4	31.7	35.8	+ 4.1
Tetraploid from Lembke [1]	3	6.41	11.78	+ 5.37	7	32.1	27.2	– 4.9	—	—	—	—

[1] Compared with Primex.

TABLE 24. *Comparison of oil content of unselected white mustard and of diploid plants with high 1000-grain weight.*

Year	Number of selected plants	1000-grain weight grams			Oil content % in dry matter		
		Unselected	Selected	Diff.	Unselected	Selected	Diff.
Selected plants with 1000-grain weights higher than 10.00 g.							
1955	33	8.59	11.30	+ 2.71	27.75	26.00	− 1.75
1956	18	8.65	10.97	+ 2.32	30.30	28.37	− 1.93
1957	24	8.08	11.56	+ 3.48	30.87	27.65	− 3.22
Selected plants with 1000-grain weights higher than 11.00 g.							
1955	18	8.59	12.06	+ 3.47	27.75	25.11	− 2.64
1956	7	8.65	11.89	+ 3.24	30.30	26.44	− 3.86
1957	15	8.08	12.24	+ 4.16	30.87	27.11	− 3.76

been discussed in detail by SCHWANITZ (1953) and he points out that in diploid *gigas*-types with large cells, the same changes in metabolism occur as in the polyploids. In the present material of diploid white mustard there is a distinct negative correlation between seed size and oil content (Table 17). In order further to illustrate this behaviour, plants with high 1000-grain weight have been selected from the previously discussed material selected for low number of seeds per pod and the oil content of these plants has been compared with that of plants from the unselected plot (Table 24). Also in this case an increase in seed size has resulted in a marked decrease in oil content. Most probably the difference is here caused by an increase in cell size.

SCHWARZE (1958) points out that probably all of the modifications in oil content may be explained by variations in the rhythm of development of the plant and in the relation between the vegetative and the reproductive phase. In earlier stages, when the plant is developing different organs, mainly carbohydrates and proteins are formed, while the storing of oil in the seed occurs mainly at the end of the reproductive phase. If this period is abbreviated, the result will usually be a decrease in oil content and therefore unripe seeds are poorer in oil but richer in proteins than ripe ones. This is evident also from the dependence of the oil-protein relation upon the place of the pod on the plant. In the soybean the protein content increases from the lowest pods upwards while the oil content decreases in the same direction (SESSONS and SCHILLER 1939).

The development is slower in the tetraploids than in the diploids and

a corresponding change may be found in the "low number of seeds" series. SCHWANITZ (1948, 1949) has shown that the relation between the vegetative and the reproductive phase of the plant is more favourable in the diploid than in the tetraploid. If the opinion of SCHWANITZ (1953) is correct, that the increase in cell volume decreases the intensity of respiration and assimilation and makes more difficult the transport of assimilates within the plant, then what here has been said indicates that when the tetraploid ripens morphologically it is in a physiologically younger stage than the diploid at the same morphological stage. This may also explain the strong negative relation between protein and oil contents always obtained (STOLLE 1954, ANDERSSON, OLSSON and LÖÖF 1956, and others) and which is especially marked in the tetraploids.

Since the low oil content in tetraploids of white mustard and turnip rape is probably caused by the increase in cell size, tetraploids with smaller cells and with a restored balance between morphological and physiological ripening may be of value since the tetraploidy increases the possibilities for heterozygosity.

VIII. DISCUSSION

The studies here reported offer a certain possibility to judge the chances for differentiating a population by selection for oil content and at the same time the selectional value of some components of yield is elucidated.

The seed yield is influenced by a number of morphological and physiological factors, each of which acts in a more or less complicated way. The actions and interactions of this factors result for the individual plant in varying numbers of pods per plant, of seeds per pod and of size of seed. These three components decide the yield of the plant, with the number of pods per plant totally dominating. When selecting it is necessary to consider this latter fact and select plants with many pods in the hope that by so doing it is possible to obtain at least some improvement of genotypically high productivity. The number of pods is, however, very strongly influenced by environment and usually there is no demonstrable correlation between the seed yield of the mother plant and that of the progeny.

In a material with normal fertility the number of seeds per pod and the weight of 1000 grains have less influence upon yield than the number of pods. On the other hand, those characters are less influenced by environment and, thus, more easily genotypically changed by selection.

TABLE 25. *Correlations between number of seeds per pod (x) and 1000-grain weight (z) in rape and turnip rape.*

Variety	Year	n	b_{zx}	r_{zx}	t^2
Winter rape 5	1958	110	− 0.013	− 0.12	1.49
„ „ 6	1958	88	− 0.019	− 0.15	1.87
Summer rape 2	1957	120	− 0.047	− 0.30	12.02***
„ „ 4	1958	103	− 0.061	− 0.38	16.84***
Winter turnip rape 1	1955	60	− 0.059	− 0.42	12.64***

It is a general opinion that the yield is more influenced by the number of seeds per pod than by the 1000-grain weight (SCHRIMPF 1954, STOLLE 1954). The importance of the seed size should not be neglected, however, and it is probable that at least a part of the increase in yield of the summer form which is obtained after crosses between summer and winter forms of rape and turnip rape is due to the increase in seed size. Compared with Regina summer rape the summer types derived from the cross summer×winter have larger seed withot a simultaneous decrease in the number of seeds per pod. Within the populations studied of rape, turnip rape and white mustard (Table 14 and 25) there is, however, a negative correlation between seed size and number of seeds per pod. The regression coefficient is low, however, and an increase in seed number results in an increased yield of seed per pod in spite of the decrease in 1000-grain weight.

The strong negative correlation between seed size and oil content observed in white mustard is traceable also in common selection material of rape and turnip rape (Table 26) but in these species it is rarely significant. In this connection it is of interest to note that summer forms of rape and turnip rape derived from the crosses between winter and summer types have both larger seeds and higher oil content than Regina

TABLE 26. *Correlations between 1000-grain weight (z) and oil content (y) in rape and turnip rape.*

Variety	Year	n	b_{yz}	$b_{yz.x}$	r_{yz}	$r_{yz.x}$	t^2 for $r_{yz.x}$
Winter rape 5	1958	110	− 0.26	− 0.16	− 0.069	− 0.043	< 1
„ „ 6	1958	88	− 0.58	− 0.50	− 0.14	− 0.12	1.19
Summer rape 2	1957	120	− 0.81	− 0.36	− 0.18	− 0.082	< 1
„ „ 4	1958	103	− 0.80	− 0.50	− 0.26	− 0.15	2.37
Winter turnip rape 1	1955	60	− 1.44	− 1.46	− 0.41	− 0.38	9.71**

summer rape and Arlo summer turnip rape, respectively. Also in comparing winter rape and winter turnip rape it is found that the former is superior both in seed size and in oil content. The negative correlation observed within genotypically fairly limited populations does not always hold true when different species or even varieties are correlated. The negative correlation within a more limited population indicates that when the size of the seed increases the increase in oil production and storage is weaker or slower than that of other components of the total dry matter, resulting in a decrease in the relative oil content. Especially in white mustard, where this negative correlation is strong, a selection for large seed without a strict simultaneous control of oil content may result in a decrease in the latter.

When discussing the influence of autotetraploidy on oil content the influence of cell size upon the latter has been dealt with and the hypothesis has been adopted that the decrease in oil content is mainly due to the increase in cell size. The negative correlation between seed size and oil content within a diploid population is also supposed to be a result of the fact that both are caused by an increase in cell size. On the other hand all variation in seed size and oil content cannot reasonably be caused by cell size, a fact which is shown by the positive relation between the two former characteristics when some genetically more widely different materials are compared. When this comparison is made between amphidiploid rape and diploid turnip rape it should be kept in mind, however, that the difference in cell size between these two is much less than that between diploid and tetraploid turnip rape.

The study of the correlations between mother plant and progeny as well as the effects of continuous selection have proved that the oil content is strongly influenced by the genotype and that it is possible to obtain considerable effects by selection.

In order to estimate the strength of the genotypical fixation of a certain character LUSH (1940, 1949) divides the variance into three portions, one of which (σ_h^2) is caused by hereditary differences, another (σ^{e^2}) by environment and the third of which (σ_j^2) "represents the joint effects of nonlinear interactions of heredity and invironment" (LUSH 1949, p. 357). The variance caused by heredity may again be divided into three parts, one being the sum of additive gene effects (σ_g^2), another by dominance effects (σ_d^2) and the third one by interactions between genes in different loci (σ_i^2). (The latter effect is usually called epistasy.) Heritability in a wider sense LUSH defines as that fraction of variance which is caused

by genotypical differences $\left(\frac{\sigma_g^2+\sigma_d^2+\sigma_i^2}{\sigma_g^2+\sigma_d^2+\sigma_i^2+\sigma_e^2+\sigma_j^2}\right)$ while "hereditary in in the narrow sense" is defined as that fraction which is caused by the additively genetic variance $\left(\frac{\sigma_g^2}{\sigma_g^2+\sigma_d^2+\sigma_i^2+\sigma_e^2+\sigma_j^2}\right)$. ROBINSON, COMSTOCK and HARVEY (1949) define heritability as "the additive genetic variance in per cent of the total variance", which corresponds to the "hereditary in a narrow sense" of LUSH.

LUSH (1949, p. 364) considers that "in a random bred population the correlation between parent and offspring include half of the genic variance and something less than a fourth of the epistatic variance". LUSH (1940) and ROBINSON *et al.* (1949) use this and calculate "the heritability as twice the parent-offspring regression".

In white mustard, which is completely cross-fertilized, the heritability should thus be twice the regression coefficient for progeny upon mother plant or in this case 38 ± 5.2 per cent. This estimate must be considered as an attempt to obtain a very approximative measure of the degree of heritability of the oil content; the determination of the regression coefficients are not very exact and further the part of hereditary variation by dominance is not considered.

From the data published by WINTER (1929) of the results obtained by 28 years of selection for oil content in maize "STUDENT" (1934) has tried to estimate the number of genes influencing the oil content. He reached the conclusion "that the oil percentage of Winter's maize was conditioned by the presence, or absence, of a number of genes at least of the order 20—40, possibly of 200—400 and not at all likely to be of the order 5—10" (*l. c.* p. 81). BOYCE (1946) has criticized "STUDENT" and considers that his calculations give too high a number of genes. Later, however, SPRAUGE and BRIMHALL (1949) have analyzed F_2 from a cross between high and low oil content and in accordance with "STUDENT" they have reached the conclusion that the number of genes causing the difference between the two strains is somewhere round 20—40. No attempt has been made to estimate the number of genes influencing the oil content of white mustard, since a much more extensive material is necessary and, furthermore, such estimates must necessarily be extremely vague, as seen *e. g.* from the rather wide limits suggested by "STUDENT". It may be reasonably safe to conclude that the number of genes influencing oil content in rape and white mustard is large.

Selection only for five generations has increased the oil content of

white mustard from 30.87 to 33.94 per cent in spite of the fact that the selection has been fairly mild, the selected material comprising as much as 20 per cent of the plants analyzed; no change in the variability has been observed. Further selection may be expected to give further advances. The increase in oil content seem to be possible without any decrease in seed yield and the selection should in the first place be sharpened to include only the ten best per cent of the material. By simultaneous selection for high oil content in genetically different populations and subsequent crosses between these and repeated selection in the cross populations it should be possible to avoid inbreeding depression and still further to accumulate genes for high oil content in a population. Already in the present material there are individual plants with an oil content of 37—38 per cent and it does not seem unreasonable to hope for an increase in the oil content of white mustard from at present about 30 per cent to a level about 40 per cent.

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SUMMARY

(1) As a rule no correlation can be demonstrated between the seed yield of an individual plant and the yield of a progeny plot. In regard to number of seeds per pod and seed size there is an evident correlation between mother and progeny.

(2) By five years continuous selection for number of seeds per pod in white mustard the number has been increased by positive selection with 1.33 ± 0.096 seeds per pod and decreased by negative selection with 1.51 ± 0.121 seeds per pod.

(3) There is a clear relation between the oil content of the mother plant and that of the progeny plot. On an average the coefficient of regression is $+0.20$.

(4) By crossing summer and winter types of rape and turnip rape a material of summer types of both species has been obtained with a higher oil yield than old varieties of the same type. In the material studied the segregation in summer : winter has followed the 3 : 1 ratio.

(5) Continuous selection during five years for oil content in white mustard has increased this content with 3.07 ± 0.323 per cent units, respectively decreased it with 2.45 ± 0.327 per cent units.

(6) The increase in oil content has been possible without any decrease

in seed yield and the "high oil content" strain has outyielded the mother variety Primex in oil per hectare with about 8 %, in average of 27 trials.

(7) There is a tendency to a decrease in 1000-grain weight with an increased number of seeds per pod and within a population of white mustard there is a definite negative correlation between 1000-grain weight and oil content.

(8) A selection for low number of seeds per pod results also in a decrease in fertility.

(9) The oil content of the white mustard seed is determined by the genotype of the mother plant and not by that of the seed itself.

(10) The increase in seed size i autotetraploids of *Brassica* and *Sinapis* results in a decrease in oil content. Probably the low oil content of the tetraploid seeds and of the large seeds of diploid white mustard are caused by the same physiological relations.

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SUPERNUMERARY TEATS IN CATTLE

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IN general three types of supernumerary teats have been observed in cattle (GIFFORD, 1934):

1. *Caudal teats*, are teats located at the rear of the normal.
2. *Intercalary teats*, are those found between the normal teats.
3. *Ramal teats*, supernumeraries that are a ramification of the normal teats.

Some of this supernumeraries may be without glands, others are orifices of small glands, and yet others may open into one of the normal glands.

A large number of investigations on the frequency of supernumerary teats in different cattle breeds have been carried out (HENNEBERG, 1904; MACKENZIE and MARSHALL, 1925; IVANOVA, 1928, 1931; GIFFORD, 1934). Some of these investigations, however, deal mainly with the possible correlation between supernumerary teats and milk production. IVANOVA (1928) concluded from data on 1385 cattle, that supernumerary teats are based on a single dominant autosomal gene, and that this gene is identical with the one that raises milk production by 15 per cent. In a comprehensive investigation by GIFFORD (1934), however, no significant evidence that extra teats being associated with milk yield was found.

At present the majority of the cattle breeders look on supernumeraries with disfavour, since the presence of extra teats detracts from the beauty of the cow, and the glands associated with the extra teats, frequently produce a small quantity of milk, thus causing irregularities and difficulties in the milking process.

Supernumerary teats are apparently found in all breeds, but the exact mode of inheritance is still unknown. It has been suggested that recessive genes are responsible for the intercalary teats but we have still no proof of this theory. In swine the number of teats represents a typical case of quantitative inheritance without dominant genes being involved (BERGE, 1949).

Own investigations

In 1956, was registered for all cows in milk testing, in the region of Hedemarken the frequency of supernumerary teats. The registration comprised a total of 6268 cows of Norwegian Red and White Cattle, and was conducted separately for the right and the left side of the udder. For the different characteristics on each side, we used the following codes:

0. No supernumerary teats.
1. One small caudal teat. (Teat less than 1 cm long, looking like a nipple).
2. One caudal teat, more than 1 cm long, but without a gland.
3. One caudal teat with a gland.
4. Two small caudal teats of the same type as under code 1.
5. One small and one somewhat larger caudal teat. (The same types as under codes 1 and 2).
6. Two caudal teats, with glands.
7. One small caudal teat and one caudal teat with a gland. (Codes 1 and 2).
8. One caudal teat more than 1 cm in length, but without a gland, and one caudal teat with a gland. (Codes 2 and 3).

The frequency of the different types of caudal teats are given in Table 1.

40.06 per cent of all cows had some type or other of supernumerary teats. This is about the same proportion as that found in previous investigations. The distribution shows that the frequency is highest for

TABLE 1. *The frequency of the different types of caudal teats on the left and the right side of the udders.*

		Codes for the left side								Total
		0	1	2	3	4	5	6	7	
Codes for the right side	0	3757	376	172	34	1				4340
	1	472	464	81	24	2				1043
	2	220	92	360	13			1		686
	3	65	19	11	82					177
	4	3	5	1	1		1			11
	5		2	5						7
	6							1		1
	7	2	1							3
	Total	4519	959	630	154	3	1	2		6268

one small nipple (13.53 per cent). Further, it will be seen that the frequency of 2 small caudal teats (code 1) is about the same as for one somewhat larger caudal teat (code 2), 7.40 and 6.25 per cent respectively. Only 0.43 per cent of all cows had more than 2 caudal teats, the frequency on the right as well as the left side, dropping steadily from code 0 to code 7.

As shown by LUSH (1954), the degree of accordance between the right and the left side may be used to test the repeatability of the characteristics. Since neither the genes, nor the dominant or epistatic effect change during the individual's lifetime, repeatability should broadly speaking be at least as large as heritability. In this case, the repeatability will be still larger because it also includes the permanent effect of the environment. With the ranking of size and number of caudal teats used in Table 1, the repeatability is 0.52.

The degree of asymmetry and the distribution of the size and number of caudal teats suggest that this is a case of quantitative inheritance. The disagreement between the left and the right side is probably mainly a question of a different manifestation of the characteristics.

Intercalary teats were found in 1.93 per cent of all cows. In this investigation no significant correlation between the number or size of caudal teats and the frequency of intercalary teats was observed. 1.8 per cent of the cows with non-caudal teats had intercalary teats. The percentage was 2.1 for cows with one small teat, 3.2 for cows with teats of the type stated under code 2, and 1.6 for cows with only one caudal teat with a gland (code 3).

34 cows, or 0.55 per cent of all cows, had one ramal teat, and none had two or more ramal teats. No correlation between the frequency of ramal teats and the number or size of caudal teats was observed.

This material was divided into 25 progeny groups, with at least 25 progeny in each. The analysis of variation (Table 2) shows that there was a significant difference in number and size of caudal teats

TABLE 2. *Analysis of variance of number and size of caudal teats for 25 progeny groups.*

Source of variation	Degrees of freedom	Mean square
Between progeny groups	24	28.190***
Within „ „	1657	5.6878
Total	1681	

between progeny groups. This differance was also significant when calculated separately for the left and for the right side of the udder.

The heritability of number and size of caudal teats was:

Separately for the left side of the udder	0.154
„ „ „ right „ „ „ „	0.170
Average of the right and the left side of udder	0.176

The coefficient of heritability found here is therefore considerably lower than the coefficient of repeatability. No significant difference in frequency of intercalary and ramal teats between progeny groups was established.

SUMMARY

The frequency of occurrence and pattern distribution of supernumerary teats, observed on 6268 cows, has been computed and reported. The percentage of cows with caudal teats was 40.06, 1.93 per cent of the cows had intercalary teats and 0.55 per cent had ramal teats.

The repeatability of size and number of caudal teats (in comparisons of the left and the right side of the udder) was 0.52. The asymmetry and the distribution of size and number of caudal teats suggest that this is a case of quantitative inheritance. The disagreement between the sides is probably a question of different manifestations of the characteristics. The coefficient of heritability for size and number of caudal teats was 0.176. There was no significant correlation between the frequency of caudal teats and that of intercalary, or ramal teats.

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CYTOGENETIC STUDIES IN THE *SERRIOLA* GROUP OF *LACTUCA*

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I. INTRODUCTION

As found by THOMPSON, WHITAKER and KOSAR (1941) and THOMPSON (1943) the three wild species *Lactuca serriola* TORNER, *L. saligna* L. and *L. virosa* L. together with a form called *L. altaica* FISCH. and MEY. and cultivated lettuce, *L. sativa* L., form a compatibility group isolated from other species of the *Scariola* section to which they belong. An account of the taxonomy of this group, which the above-mentioned authors called the *serriola* group, will be given in another paper (LINDQVIST, 1960 c). Cytological and genetical investigations involving species of this group have been carried out by a number of authors. The results of these investigations, which will be discussed in appropriate connections in this paper, may be summarized as follows.

The chromosome number of all the species of the *serriola* group is $2n=18$, but other species of the *Scariola* section have the somatic numbers 16 or 34. Among the wild species of the *serriola* group only diploid forms are known, but autotetraploid forms of *L. sativa* have been produced by the colchicine method (THOMPSON and KOSAR, 1938).

Hybrids between *L. sativa* and *L. serriola* are easily obtained. They have normal meiosis and are highly fertile. Hybrids between *L. sativa* and *L. virosa* and between *L. saligna* and *L. serriola* are difficult to obtain, have an irregular meiosis and are highly sterile. It is obvious that *L. sativa* is very closely related to *L. serriola*, but there is little information about its relationship with the other wild species in the *serriola* group. This investigation was undertaken in order to gain further information about this question. It has entailed observations on general morphology, cytology and fertility of pure lines within the different species and on intraspecific and interspecific hybrids.

II. MATERIAL AND METHODS

The material consisted of lines of *L. sativa, L. serriola, L. saligna* and *L. virosa.* These originated from commercial varieties, from wild growing populations or from seed samples obtained from botanic gardens. Detailed accounts of the lines will be given separately for each species.

Seedlings were normally raised in a heated greenhouse. Seeds of the wild species and some hybrids, however, germinated slowly and unevenly when sown in the ordinary way, and better results were obtained, if they were allowed to germinate on filter papers in the laboratory and the seedlings were pricked out into a seed compost immediately after germination. The plants were, as far as possible, grown to maturity in a cold greenhouse, but as the greenhouse space was limited it was often necessary to grow plants out of doors which proved very hazardous as much damage was caused by various fungi, particularly *Botrytis cinérea* PERS.

The floral mechanism in *L. sativa* and related species ensures a high degree of self-fertilization. A detailed account of this mechanism has been given by JONES (1927). The flower heads are open only during the morning, the times when they open and close being determined by the temperature. Whilst the flower heads are open the pistil grows through the tube formed by the five anthers and emerge with the stigmatas covered in pollen. After a short time the petals close and do not open again. Thus new flower heads open each day.

The flower heads are visited by various insects, but, as the pistils are already covered with pollen when they break through the anther tube, and as they are exposed to the risk of cross-pollination only for a very short time, cross-fertilization is exceptional. DURST (1930) found that cross-fertilization in lettuce is a rare event. THOMPSON, WHITAKER and BOHN (1958) found that the amount of cross-pollination between different lettuce varieties grown in adjacent rows, was usually about 1 %. When plants of one variety were completely surrounded by plants of another variety, the amount of cross-pollination varied between zero and 18 % for individual plants, the average being 2.87 %. They found that most of the contamination occurred in the direction of the prevailing wind and their explanation is that the movement of pollen carrying insects is controlled by the wind. They found significant differences in the amount of cross-pollination at different times during the flowering period. WATTS (1958) found that the amount of spontaneous crossing was less than 1 % during the summer months, but that it increased to

11.5 % in plants which flowered late in the season. WATTS (*l.c.*) attributes this to a seasonal increase in the hover-fly population.

Isolation of flowering branches by bagging has been the rule during the present investigation, not only for the purpose of preventing cross-pollination, but also as a protection against *Botrytis* spores and the Lettuce Seed Fly, *Cortophila gnava* MEIG. The method described by ERNST-SCHWARZENBACH (1936) has been used for crossing, *i.e.* the anther tubes were removed before the pistils had grown through, and any pollen that had adhered to the stigmata was washed away with water.

Pollen samples were taken after the pistils had grown through the anther tube. The pollen-covered pistils were dipped into a drop of a mixture of aceto-carmine and glycerine or lactophenol and cotton blue. If possible at least three flower heads per plant were used and a minimum of 200 pollen grains were counted. Female fertility was determined by counting the number of ripe achenes and undeveloped ovules on 20 fruiting heads per plant. In forms with the character reflexed involucre (LINDQVIST, 1957) great care had to be taken to avoid loss through shedding. In such forms fruiting heads were chosen in which the involucral bracts were still erect although the achenes were ripe. As the achenes of *Lactuca* are usually referred to as seeds, this will be done also in this paper.

Somatic chromosomes were studied in root tip squashes after pretreatment in oxyquinoline according to the method of TJIO and LEVAN (1950). The slides were made permanent by mounting in Euparal after removing the cover glasses with the aid of dry ice. Measurements were made on camera lucida drawings at a magnification of 5800. Each chromosome was drawn in a central position in the optical field.

Meiosis was studied in PMC. Buds were fixed in Carnoy, 6 : 3 : 1, hardened in absolute alcohol overnight and stored in 70 % alcohol. After hydrolysis for six minutes in N HCl and staining in Feulgen, the pollen loculi were teased out under a dissecting microscope and squashed carefully under the cover glass. The Feulgen technique did not always give satisfactory staining, but this could often be rectified by squashing in a drop of 1 % propionic carmine. As far as possible observations were made on fresh slides, but when necessary, slides were made permanent by mounting in Euparal.

III. EXPERIMENTAL RESULTS

1. L. sativa

A. Pure lines

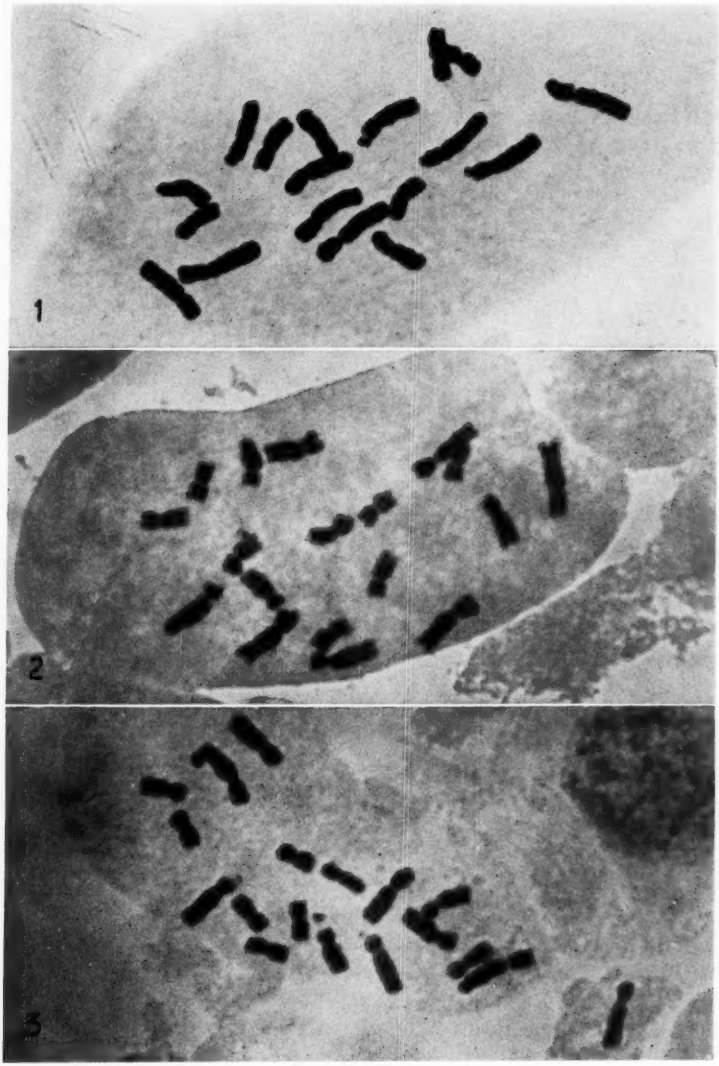
a. *Origin and characteristics*

The lines of *L. sativa* originate partly from cultivated varieties obtained from various seed firms and partly from forms obtained from botanic gardens and having the appearance of primitive types.

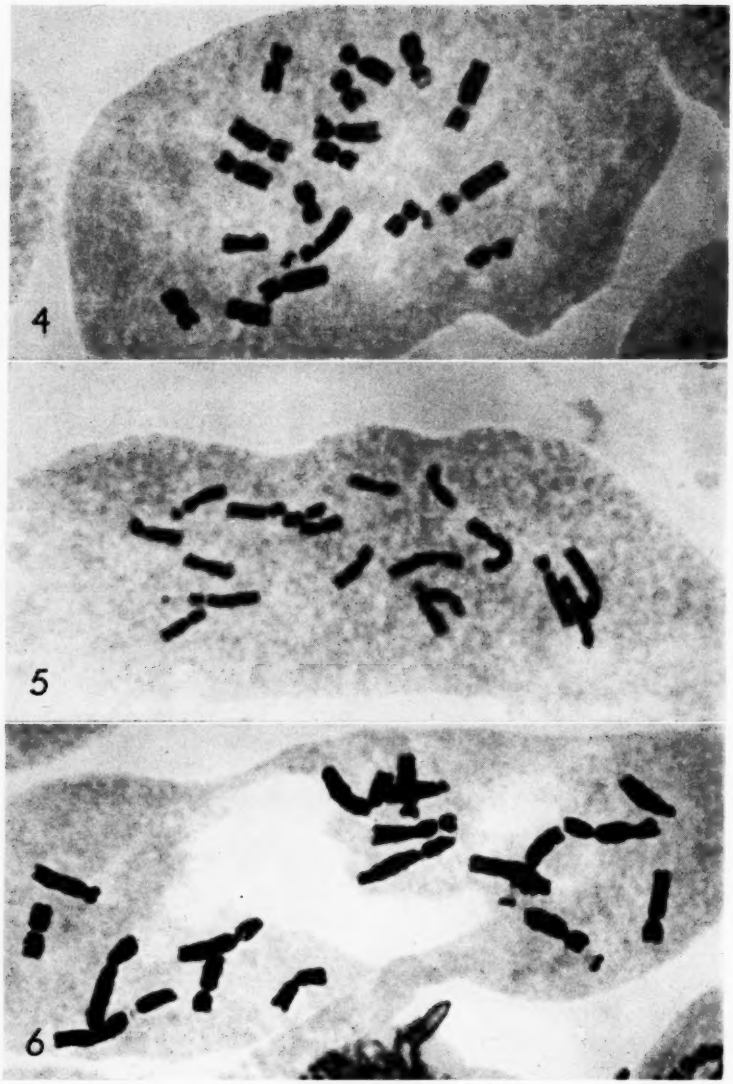
Because of the prevalence of self-fertilization in lettuce, commercial varieties are extremely uniform and, without doubt, highly homozygous. The differences between varieties are, as a rule, very conspiciuous, so that admixtures or spontaneous hybrids are recognized without difficulty. The lines, which have been propagated by single plant progenies year after year, are referred to by an abbreviation of the name of the variety from which they originate. There is considerable confusion concerning names of lettuce varieties, and lists of synonyms have been published by several authors, the most comprehensive by MORSE (1930). The following list contains, wherever possible, the names used by MORSE as well as the names under which the varieties were obtained.

Abbreviation	Name under which received	Name used by MORSE
Asp.	Asparagus lettuce	Asparagus Cos
Oakl.	—	Baltimore Oak Leaf?
Eichenbl.	Eichenblättriger	Philadelphia Oak Leaf?
Paris	Paris White Cos	White Paris Cos
G.W.Cos	Giant White Cos	Giant White Cos
R.rouge	Romaine rouge d'hiver	Red Winter
K.driv	Kejsar driv	Emperor Forcing
Berl.	Berliner	Blonde de Berlin
Maik.	Maikönig	May King
Merv.	Merveille des quatre saisons	Marvel or Red Besson
Laur.	Laurenzianer	Thorburn's Giant Cabbage?
Wond.	Webb's Wonderful	New York
Tezier	Tezier	—
Rosette 48	—	—
T+g	—	—

The line Oakl., which originates from plants found as admixtures in another variety, agrees well with MORSE's Baltimore Oak Leaf and Eichenbl. with Philadelphia Oak Leaf, but material has not been available for direct comparison. The variety Laurenzianer is a yellow seeded variety with spotted leaves which very well fits the description given by MORSE for Thorburn's Giant Cabbage. It is not identical with Casard,



Figs. 1—3. Somatic chromosomes of *L. sativa*. — Fig. 1. Berl. Fig. 2. R.rouge. Fig. 3. SP.



Figs. 4—6. Somatic chromosomes. — Fig. 4. *L. serriola*, line Sc.L. Fig. 5. *L. saligna*, line Sal.R. Fig. 6. *L. virosa*, line Vir.L.

for which the name Laurenzianer has also been used (*cf.* BREMER, 1931, p. 481). The last two lines in the list have been selected from crosses between varieties of *L. sativa.*

A detailed account of the primitive forms of *L. sativa* will be found in another paper (LINDQVIST, 1960 c). They were received from botanic gardens under the names shown below.

Abbreviation	Origin	Name
Altaica	Kew, England	*L. altaica* FISCH. and MEY.
LC	Copenhagen, Denmark	*L. livida* BOISS. et REUT.
SL	Lund, Sweden	*L. saligna*
SP	Palermo, Italy	*L. sagittata*
SB	Brno, Czechoslovakia	*L. virosa*
SM	Modena, Italy	*L. dregeana* D.C.
ST	Torino, Italy	*L. serriola*

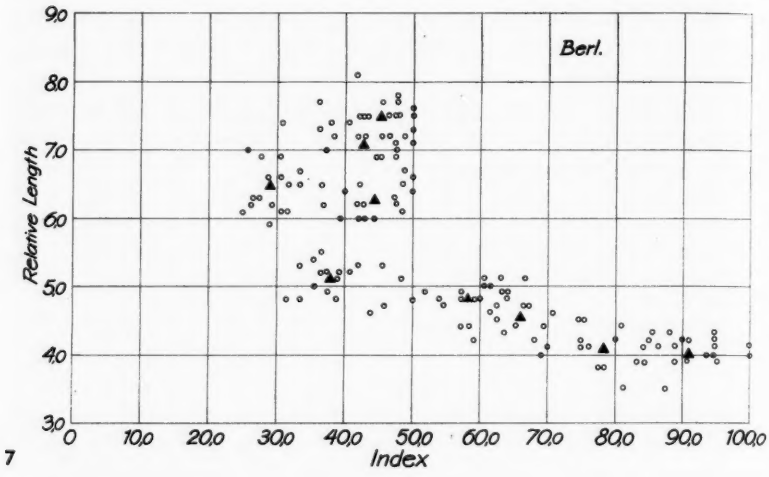
From the beginning all these lines have been uniform and true-breeding. It should be mentioned, however, that other samples gave a mixture of forms of primitive *L. sativa* type. The progenies of a number of such plants were tested and found to breed true, which indicates that the original populations were mixtures of different types, not segregating populations. There is reason to believe that the *L. altaica* of THOMPSON, WHITAKER and KOSAR (1941) was similar to these primitive forms of *L. sativa* although probably not identical with the line Altaica used in this investigation.

b. *Cytology*

Chromosome morphology: — Somatic chromosomes have been studied in six lines of cultivated lettuce, namely Asp., R. rouge, Paris, Berl., K. driv and Oakl., and in six primitive forms, namely Altaica, SL, SP, SM, SB and ST. Figs. 1—3 illustrate the chromosomes of three of these lines, Berl., R. rouge and SP.

The nine chromosome pairs show great variation with regard to overall length and with regard to position of the centromeres. The longer chromosomes have subterminal or submedian centromeres, whereas the shorter chromosomes have almost median centromeres. Two of the chromosome pairs carry satellites and these pairs are easy to distinguish from each other, one pair being fairly short with submedian centromere, the other considerably longer with subterminal centromere. The remaining seven pairs cannot be identified with certainty, but three of these pairs are longer and the other four are shorter than the shorter SAT-pair.

Apart from a variation in the size of the satellites, which will be de-



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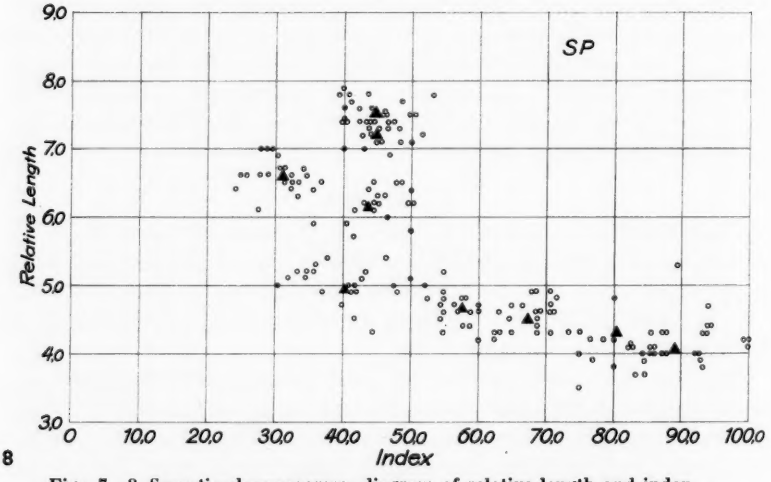

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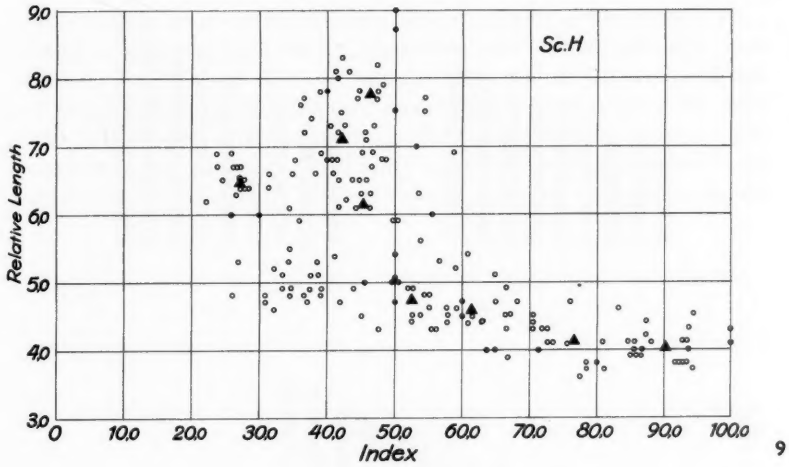
Figs. 7—8. Somatic chromosomes; diagram of relative length and index. — Fig. 7. *L. sativa*, line Berl. Fig. 8. *L. sativa*, line SP.

scribed below, no differences were observed between the chromosomes of the twelve lines which were studied. A critical comparison was made between two of the lines by means of measurement. The length of each chromosome arm was measured in 9 cells of the line Berl. and in 10 cells of the line SP. From these data 'relative length' and 'index' were



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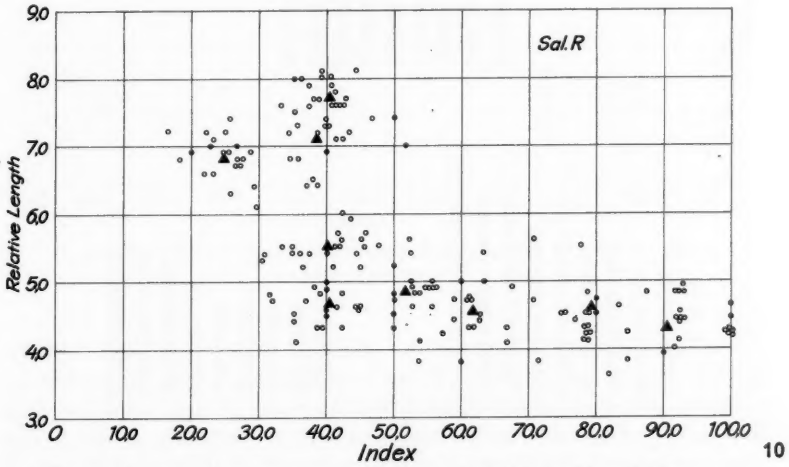

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Figs. 9—10. Somatic chromosomes; diagram of relative length and index. — Fig. 9. *L. serriola*, line Sc.H. Fig. 10. *L. saligna*, line Sal.R.

calculated for each chromosome (*cf.* TJIO and HAGBERG, 1951). The relative length is the overall length of the chromosome expressed as percentage of the total length of all the chromosomes in the cell. The index is the length of the shorter arm expressed as percentage of the length of the longer arm.

In Figs. 7 and 8, relative length and index have been plotted against each other for the two lines separately. An attempt was made to identify the 9 pairs and averages were calculated on the basis of this classification. They are represented by black triangles in Figs. 7 and 8 and have been used in Figs. 12 and 13 for a diagrammatic presentation of the two chromosome sets. It is clear from these diagrams that the chromosomes of the two lines are, if not identical, at least extremely similar.

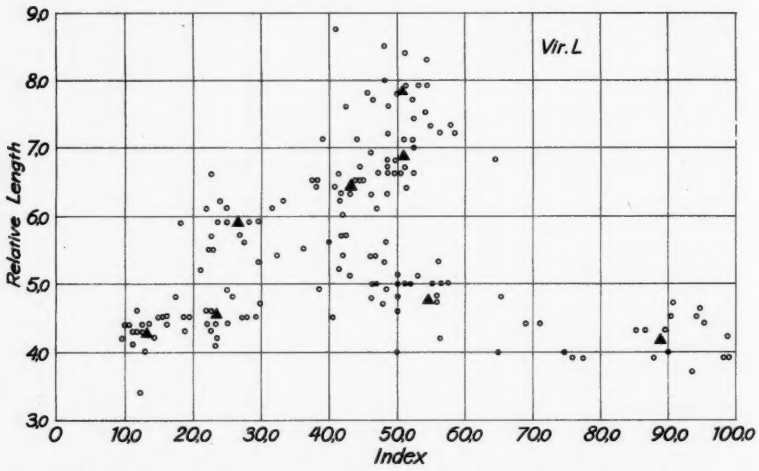
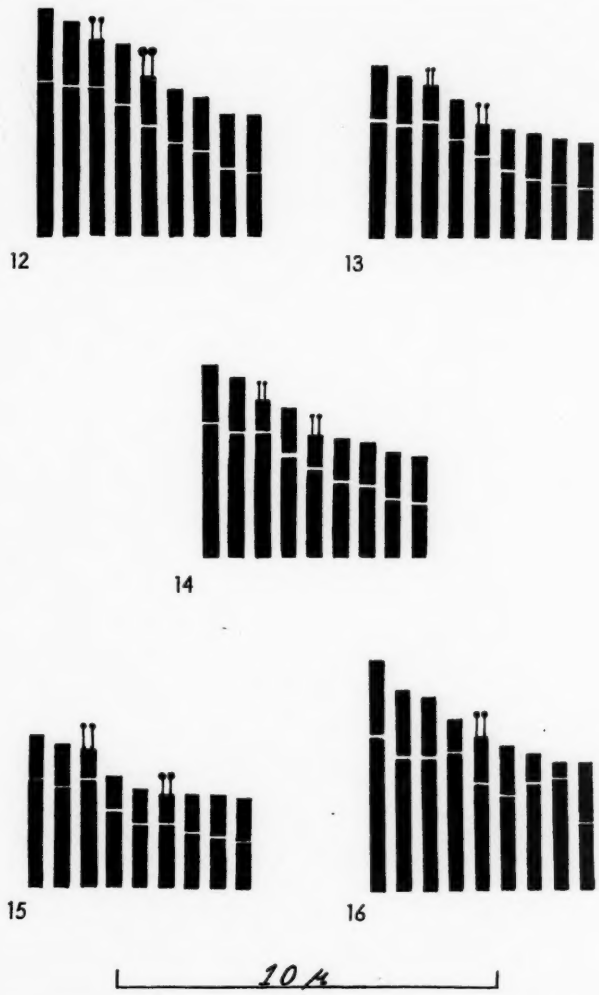


Fig. 11. Somatic chromosomes; diagram of relative length and index. *L. virosa*, line Vir.L.

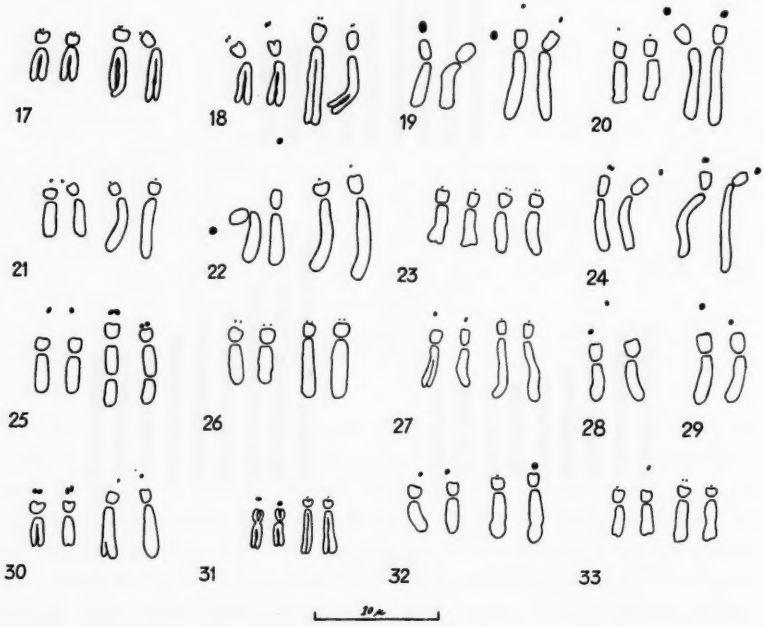
Distinct differences between the different lines were found with regard to size of the satellites. These were too small to be measured accurately and were instead divided into three size groups, small, medium and large, which have been designated with the letters s, m and l. This does not mean that only three distinct types of satellites were present in the material. The variation was continuous and the classification therefore in some cases rather arbitrary. Satellite size was, however, found to be constant for cells of the same plant and plants of the same line.

The 12 lines have been classified below. The shorter satellite pair has been designated as SAT I and the longer pair SAT II. Camera lucida drawings of the two satellite pairs from some of the lines are presented in Figs. 17—24.



Figs. 12—16. Idiograms. — Fig. 12. *L. sativa*, line Berl. Fig. 13. *L. sativa*, line SP. Fig. 14. *L. serriola*, line Sc.H. Fig. 15. *L. saligna*, line Sal.R. Fig. 16. *L. virosa*, line Vir.L.

Line	SAT I	SAT II	Fig.
Asp.	s	s	17
R.rouge	m	s	18
Paris	s	s	—
Berl.	l	m	19
K.driv	s	l	20
Oakl.	s	m	—
Altaica	m	s	21
SL	l	s	22
SP	m	s	—
SM	s	s	23
SB	s	s	—
ST	l	l	24



Figs. 17—33. Satellite chromosomes. — Figs. 17—24. *L. sativa.* Fig. 17. Asp. Fig. 18. R.rouge. Fig. 19. Berl. Fig. 20. K.driv. Fig. 21. Altaica. Fig. 22. SL. Fig. 23. SM. Fig. 24. ST. — Figs. 25—27. *L. serriola.* Fig. 25. Sc.L. Fig. 26. Sc.H. Fig. 27. Sc.aug.All. — Figs. 28—29. *L. virosa.* Fig. 28. Vir.T. Fig. 29. Vir.J. — Figs. 30—31. *L. saligna.* Fig. 30. Sal.R. Fig. 31. Sal.C. — Figs. 32—33. F_2-plants. Fig. 32. Sc.H×Sc.L. Fig. 33. Altaica×Sc.H.

Meiosis: — Because of the great morphological and physiological variation within *L. sativa* it was considered necessary to include several different lines in the investigation in order to get a picture of the variation in meiotic behaviour. Only one or a few plants of each line could therefore be studied, which means that little importance can be attached to differences between the individual lines, particularly as the fixations were made at different times and under different conditions. Meiosis was studied in twelve lines of *L. sativa*, seven lines from cultivated varieties and five from primitive types. In addition two off-types, 'cos' and 'mut', found in the variety Tezier were examined. These off-types will be described in another connection.

Various meiotic abnormalities, such as univalents at I M (Fig. 34), difficulties in separation at I A, lagging chromosomes and bridges at I A (Fig. 36) and in some cases also at II M (Figs. 39 and 40) and II A, were observed fairly frequently. The percentages of cells with such abnormalities have been recorded in Table 1. Structures similar to inversion bridges were sometimes observed at I A, but intermediate stages were seen between such cases and normal bivalent separation. A detailed classification of the abnormalities would of necessity have been arbitrary and was not attempted. The nature of the bridges will be discussed below.

In this investigation the average number of ring bivalents per cell has been used as an approximate measure of the chiasma frequency. All bivalents with one or more chiasmata in both arms, were counted as rings and those with one or more chiasmata in one arm only, as rods. The correlation between number of chiasmata per cell and number of ring bivalents per cell was good, as shown by the following data, obtained from four different hybrids:

Mean X-ma frequency	Av. no. of ring biv.	No. of cells	Coeff. of regression
19.8	7.4	77	0.878
19.0	7.4	52	0.876
15.5	4.9	81	1.042
14.6	4.6	53	0.962

The number of chiasmata per bivalent was ascertained also for the two lines K.driv and R.rouge and found to be 1.88 (34 cells) and 2.07 (62 cells) respectively. Bivalents with two or three chiasmata in the same arm were not rare.

PMC with univalents (Fig. 34) were found in all lines. The frequency of such cells was usually low, but reached considerable proportions in

TABLE 1. *Meiosis*

Lines or hybrids	No. of plants	I M						
		Pairing						
		No. of PMC with					Number of cells	% PMC with univ.
		9 II	8 II 2 I	7 II 4 I	6 II 6 I	< 6 II		
Lines								
Asp.	1 (G)	116	2				118	1.7
Oakl.	1 (G)	91	6				97	6.2
Berl.	1 (G)	153	7	1			161	5.0
Maik.	1 (G)	61	1				62	1.6
K. driv	1 (G)	34	2				36	5.6
R. rouge	1 (G)	63	2	1			66	4.5
Tezier	2 (G)	63	4				67	6.0
„ cos	3 (G)	146	50	7	2		205	28.8
„ mut	1 (F)	90	7	1			98	8.2
Altaica	1 (G)	112	2				114	1.8
LC	2 (G)	118	18				136	13.2
SL	1 (G)	39	7				46	15.2
SB	1 (G)	103	8				121	6.6
SP	1 (G)	76	20				96	20.8
SP	1 (G)	86	5				91	5.5
Hybrids								
Asp. × R. rouge	1 (F)	76	23	4			103	26.2
Asp. × K. driv	1 (F)	96	3	1			100	4.0
Berl. × Oakl.	1 (G)	140	1				141	0.7
Berl. × Wond.	1 (G)	124					124	0.0
Altaica × SL	1 (G)	78	9	1			88	11.4
Altaica × LC	2 (G)	54	4				58	6.9
SL × SP	1 (F)	34	9	4			47	27.7
SB × SL	1 (F)	9	12	8	2	6	37	75.7
SL × SM	1 (F)	41	9	1	—	2	53	22.6
K. driv × Altaica	1 (F)	131	1				132	0.8
G. W. Cos × Altaica	1 (G)	89	2				91	2.2
LC × Oakl.	1 (G)	32					32	0.0
Berl. × LC	1 (G)	88	3				91	3.3
Asp. × SL	1 (F)	142					142	0.0
G. W. Cos × SL	1 (G)	65					65	0.0
Oakl. × SP	1 (G)	65					65	0.0
Wond. × SL	1 (G)	99					99	0.0

(G) = Grown in the greenhouse (F) = Grown on the field

in L. sativa.

I M		I A+I T			II M			II A+II T				
Ring-rod II												
Average number of ring biv. per cell	Number of cells	% cells with bridges	% cells with lagg. chrom.	Number of cells	% cells with bridges	% cells with lagg. chrom.	Number of cells	% cells with bridges	% cells with lagg. chrom.	Number of cells	% good pollen	% seed setting
1.7	—	0	0	39	0	0	20	0	0	17	100.0	37.6
6.2	23	0	0	15	0	0	14	0	0	7	95.2	84.7
7.2	33	0	0	60	0	0	5	—	—	—	—	—
7.1	61	2.2	0	46	0	0.8	122	4.8	0	21	98.0	87.8
5.9	34	5.4	0	37	0	0	156	0	0	49	99.0	78.4
7.0	66	0	0	15	—	—	—	—	0	31	—	84.0
6.2	42	0	4.9	41	0	9.3	54	2.9	11.4	35	95.2	39.0
5.6	133	5.2	11.7	77	1.2	14.0	86	1.8	15.8	57	87.1	71.2
6.7	54	4.2	12.5	24	0	3.0	33	3.7	25.9	27	90.4	—
7.7	46	0	0	20	0	0	7	—	—	—	97.0	—
6.1	113	0	0	18	0	4.7	43	3.6	7.3	55	95.0	—
6.9	8	0	11.8	17	0	3.0	33	0	0	13	100.0	76.5
6.8	103	3.6	0	28	0	1.2	81	0	0	6	—	16.6
6.3	76	11.1	0	9	0.8	7.7	130	0	0	32	98.0	49.2
8.0	86	2.1	6.3	48	0	0.7	139	1.2	0	86	—	—
5.5	76	45.1	7.8	51	7.0	4.2	71	0	0	24	80.9	63.7
8.2	96	1.9	1.9	53	0	4.3	93	0	0	90	80.8	70.9
7.3	60	—	—	—	0	2.9	35	2.0	2.0	49	98.5	82.2
7.7	36	0	0	4	—	—	—	—	—	—	100.0	87.9
6.9	60	9.5	9.5	42	1.9	1.9	52	4.9	0	41	50.4	74.9
5.3	38	0	1.5	65	0	1.4	70	0	1.8	55	93.5	—
6.4	29	35.3	17.6	34	2.6	15.4	39	0	9.4	32	84.7	—
6.0	9	68.8	18.8	16	0	0	27	14.3	42.9	7	55.7	—
5.9	29	31.8	4.5	22	0	0	30	5.0	10.0	20	—	—
7.4	131	3.3	13.3	30	0	1.5	68	—	—	—	99.0	84.4
7.2	17	7.6	1.0	105	0	2.8	71	1.3	1.3	75	93.5	96.2
6.5	4	0	0	9	—	—	—	0	0	11	97.1	—
7.3	80	0.8	0	13	2.0	0	49	0	0	41	99.6	95.2
8.0	142	16.7	0	12	—	—	—	—	—	—	—	—
7.1	26	33.3	0	15	0	1.3	79	5.6	0	36	44.2	81.6
6.7	64	—	—	—	0	0	93	0	0	33	54.3	89.2
6.8	99	2.3	0	44	2.2	15.6	45	0	0	26	77.2	—

TABLE 2. *Pollen fertility in L. sativa.*

Lines or hybrids	Greenhouse									Field							
	% good pollen									% good pollen							
	100	90	80	70	60	50	40	30	Average	100	90	80	70	60	50	40	Average
Lines																	
Asp.	15								98.3	16							97.1
R. rouge	13								96.5	3	1	2	1				82.2
Paris	8								97.7								—
G. W. Cos	5								96.6								—
Oakl.	14	1							96.8	3	—	1					92.1
Eichenbl.	3								97.3								—
Berl.	14								97.9	10	2	—	—	1			90.1
K. driv	14								98.3	6							97.3
Laur.	10								98.1	3							98.0
Maik	10								97.7	4							95.6
Tezier	8	2							91.8	3							91.1
„ cos	11	4	—	—	1				90.2	3	8	1					86.6
„ mut	2	1							88.6	1							90.4
Wond.	3								96.3								—
Rosette 48	7								97.5								—
T + g	2								97.2								—
Altaica	12	3	3						92.2	4	3	1	1				85.5
LC	13	1							95.1	6							95.5
SL	13								97.7	7							94.1
SB	11								97.3	3							96.0
SP	18	2	4	1	1				90.7	11	—	—	—	1			91.3
SM	11								98.4								—
ST	6								96.7								—
Hybrids																	
Berl. × Asp.	3								97.9		3	2					79.3
Asp. × K. driv											2	1					78.6
Asp. × R. rouge											1	1	1				76.2
Oakl. × Asp.	2								97.1		2	1					79.4
Berl. × Paris										1							100.0
Berl. × Oakl.	3								98.7								
Berl. × Wond	3								99.3	1							97.1
Paris × Eichenbl.										2							100.0
Paris × Oakl.										1							97.3
R. rouge × Oakl.										4							99.0
Maik. × Rosette 48	2								95.4	3							96.8
T + g × Rosette 48										1							96.8
Eichenbl. × T + g										1							99.5

Lines or hybrids	Greenhouse									Field							
	% good pollen									% good pollen							
	100	90	80	70	60	50	40	30	Average	100	90	80	70	60	50	40	Average
Hybrids																	
Altaica × LC	6								95.7	3							95.4
Altaica × SL					1	1	1		45.3		1	—	—	5	1		58.9
Altaica × SP												1					77.8
LC × SL			1	1					71.4								
SL × SP	4	—	2						88.9	1	2	1					84.1
SB × SL	1	2							85.6		2	—	1	1			69.9
SL × SM				2	1				60.8								
Altaica × Asp.									—			2					74.2
Maik. × Altaica	3								97.2	2							97.8
Laur × Altaica									—	1							99.0
K. driv × Altaica									—	4							98.9
G.W. Cos × Altaica	5								94.7								—
Berl. × LC	6								99.2	1	1						91.6
LC × Oakl.	2								96.0								—
Berl. × SP									—		3	1	1				80.4
Oakl. × SP					3				55.1							2	46.6
SB × Maik.									—						1		58.6
Asp. × SL									—		1	1	3				69.6
Berl. × SL																1	45.5
K. driv × SL															2	1	52.6
Wond. × SL			2	1					74.4				2				64.9
G.W. Cos × SL				1	—	5			48.8								

some of the primitive forms and in the cos off-type of Tezier. The univalents were usually large, which suggests that the non-pairing was mainly confined to the larger chromosomes of the complement. In plants with a high proportion of univalents the chromosomes were often less contracted than normal.

c. *Fertility*

Pollen fertility: — Data on pollen fertility in a number of lines of *L. sativa* have been collected over a number of years partly on plants grown in the greenhouse and partly on plants grown in the field. The results are presented in Table 2.

The effect of the environment on pollen fertility has been studied in material which consisted mainly of *L. sativa* lines, but included some lines of *L. serriola*, *L. saligna* and *L. virosa*. As a rule three plants were

sampled of each line. In 1957 a comparison was made between the pollen fertility of 26 lines in the greenhouse and on the field. The average pollen fertility in the greenhouse was found to be 97.19 % and on the field 95.34 %. The difference, 1.85 %, had a standard error of 0.435 % and was thus highly significant ($P<0.001$). During the years 1955 to 1958 the average pollen fertility in the greenhouse of thirteen different lines was found to be 96.41 %, 95.66 %, 96.55 % and 95.50 %. The highest average was thus obtained in 1957 and the lowest in 1958, but the difference, 1.05 ± 0.924 is not significant ($P=0.2$—0.3). Thirteen lines gave in 1954 on the field an average pollen fertility of 88.14 % and in 1957 95.05 %, and the average of six lines was in 1956 94.00 % and in 1957 98.10 %. Neither difference, 6.91 ± 3.922 and 4.10 ± 2.178, is significant, because of large standard errors.

These results indicate that plants grown in the greenhouse as a rule have better pollen fertility than plants grown in the field, the difference being significant even for 1957, when conditions were very favourable for the field plants. There appears to be little difference between different years with regard to pollen fertility under greenhouse conditions, whereas results obtained under field conditions are much more variable.

In Table 2 data from greenhouse plants and field plants have been kept separate, but data from different years had to be added together in order to economise with space. This must be taken into consideration when comparing the results, particularly with regard to field plants. The pollen fertility was on the whole very good, as is to be expected in pure lines of an autogamous species. A certain weakness is, however, apparent, particularly in some of the primitive forms and in the off-types of Tezier. This is presumably connected with the meiotic irregularities described above.

Seed setting: — Seed setting in cultivated lettuce is greatly influenced by environmental factors. Damp weather at the time of ripening has a very detrimental effect on many varieties, the main reason being susceptibility to attacks of fungi such as *Bremia lactucae* REG. and *Botrytis cinerea*. The data on seed setting presented in Table 3 are therefore based entirely on greenhouse material.

The influence of different years on seed fertility was studied on material consisting of eight lines grown in the greenhouse in the years 1956—1958. Seed fertility counts were made on three plants from each line each year, and the averages obtained were 71.0 %, 70.0 % and 78.2 %. The differences between the different years are not statistically significant. The greatest difference, *i.e.* 1958—1957, is 8.2 ± 4.50 ($P=0.1$—

TABLE 3. *Seed setting in L. sativa. Greenhouse plants.*

Lines or hybrids	% seed setting										
	100	90	80	70	60	50	40	30	20	10	Average
Lines											
Asp.			2	2	4	2	—	1	1		61.6
Berl.		5	2								92.8
Oakl.		4	8								88.0
K. driv			4	5							80.2
Laur.		3	2	1							85.5
Rosette 48		3	4	2							85.7
Wond.					2						65.6
Maik.		3	4								88.9
R. rouge			4	3	—	4					71.6
Paris					1						69.3
Tezier cabb.						1					55.9
„ cos			2	1	—	2					69.6
„ mut			1		1						72.5
Giant W. Cos				1							72.5
Altaica			2	7	3						74.3
LC			1	2	4	3	—	1			63.0
SL		1	1	4	3	1					73.6
SP		1	1	—	1	3	3				61.9
SB				1	1	—	—	1	1	2	39.6
SM			2	1							82.9
Hybrids											
Berl. × Asp.		2	1								90.3
Berl. × Oakl.			3								86.3
Berl. × Wond.		1	2								88.9
Oakl. × Asp.			1	1							84.0
Maik. × Rosette 48		1	1								89.4
SL × SP			3								84.0
Altaica × LC		1	1								88.3
Altaica × SL				2	1						71.8
LC × SL					1						64.6
Maik. × Altaica		3									95.6
G. W. Cos × Altaica		2	1								94.2
Berl. × LC		4	2								91.9
Oakl. × SP		1	2								89.6
G. W. Cos × SL			1	—	2						67.7

0.2). Thus, differences in seed setting between different years do not appear to be serious under greenhouse conditions. Results from different years have therefore been added together in Table 3. The data in this table show that seed setting in *L. sativa* is rarely complete. In some lines, particularly among the primitive forms, seed fertility can be quite low.

B. Hybrids within L. sativa

a. *Description of hybrids*

A large number of crosses have been made between different lines of *L. sativa* and no evidence of incompatibility has been found. The F_1-hybrids developed normally without showing any conspiciuous hybrid vigour. Measurements of cotyledons and young leaves showed, however, that the hybrids often developed more rapidly than the best of the parents which suggests a heterosis effect on the early stages of development. The later vegetative development is greatly influenced by the photoperiodic reaction of the plant. Characters such as size of fully grown leaves, height of stem, time of flowering etc. are therefore not suitable for heterosis studies as the interpretation of the results would be rendered difficult by the effect of genes for photoperiodic reaction.

b. *Cytology*

Meiosis: — Data on meiosis in a number of hybrids within *L. sativa* have been recorded in Table 1. The same kinds of irregularities, which were found in the pure lines, were encountered in varying degrees also in the F_1-hybrids. The most serious disturbances were found in Asp.× R.rouge and in some of the hybrids between primitive forms. A PMC with very bad pairing, 3 II+12 I, is shown in Fig. 35, and a typical case of difficult separation at I A is illustrated in Fig. 37. Bridges were observed at I A, which were very similar to inversion bridges (Fig. 38). Some persisted until II M and II A (Fig. 41). Hybrids between cultivated and primitive forms showed on the whole less irregularities than the parents and than hybrids between different cultivated forms and hybrids between different primitive forms.

c. *Fertility*

Pollen fertility: — Data on pollen fertility of hybrids within *L. sativa* are found in Table 2. As in the case of the pure lines, data from greenhouse plants and field plants have been recorded separately. Many of

TABLE 4. *Seed setting. Comparison between F_1 hybrids and parent lines in the greenhouse.*

Hybrids	F_1		Parent lines					Diff. F_1 and	
	Seed setting	No. of plants	Seed setting	No. of plants	Seed setting	No. of plants	Average	Average of parents	Best parent
L. sativa × *sativa*									
Berl. × Oakl.	86.3	3	88.2	3	99.4	1	93.8	− 7.5	− 13.1
Berl. × Asp.	90.3	3	80.3	3	95.4	3	87.8	+ 2.5	− 5.1
Oakl. × Asp.	84.0	2	80.3	3	88.5	3	84.4	− 0.4	− 4.5
Maik. × Rosette 48	89.4	2	88.6	3	93.1	3	90.9	− 1.5	− 3.7
LC × SL	64.6	1	76.5	1	79.4	3	78.0	− 13.4	− 14.8
Altaica × LC	88.3	2	68.9	3	73.8	3	71.4	+ 16.9	+ 14.5
Altaica × SL	71.8	3	73.8	3	81.2	3	77.5	− 5.7	− 9.4
Berl. × LC	93.6	3	53.0	3	95.4	3	74.2	+ 19.4	− 1.8
G. W. Cos × Altaica	94.2	3	72.5	1	73.8	3	73.2	+ 21.0	+ 20.4
L. serriola × *serriola*									
Sc. H × Sc. L	89.0	3	93.0	3	94.0	3	93.5	− 4.5	− 5.0
L. sativa × *serriola*									
Berl. × Sc. L	89.0	1	86.9	3	95.4	3	91.2	− 2.2	− 6.4
R. rouge × Sc. S. Afr.	88.4	3	42.3	3	65.2	3	53.8	+ 34.6	+ 23.2
Paris × Sc. J	97.0	3	69.3	1	90.5	3	79.9	+ 17.1	+ 6.5
Altaica × Sc. H	97.4	2	77.1	3	93.0	3	85.1	+ 12.3	+ 4.4
LC × Sc. H	90.0	2	68.9	3	93.0	3	81.0	+ 9.0	− 3.0

the hybrids are partly pollen sterile, but the significance of this is somewhat obscured by the occurrence of partly pollen sterile plants also in the pure lines. However, in many of the hybrids all the plants have a pollen fertility of less than 90 %, which is never the case in any of the lines. Further, all plants with partial pollen sterility — with the exception of one plant of the hybrid Berl. × LC — occur in crosses, in which one of the following four lines is involved: Asp., SL, SP and SB. These lines have produced F_1-hybrids with normal pollen fertility only when crossed with each other. An exception is the greenhouse plants of the two hybrids Berl. × Asp. and Oakl. × Asp.

The results will be discussed later in this paper together with other data, and it will be shown that the partial pollen sterility is correlated with abnormal segregation of certain genes.

Seed setting: — Seed setting in a number of F_1-hybrids and their parent lines was compared, partly in the greenhouse and partly under

rather unfavourable conditions out of doors. Some hybrids between *L. sativa* and *L. serriola* were included in these comparisons. The data in Table 5 show that the F_1-hybrids in the field had a much better seed setting than the parents, which all gave very poor and surprisingly similar results. No data are available for the line Asp., which flowered rather late and failed to set any seeds at all. The data in Table 4 show that there is a tendency for the F_1-hybrids to have better seed setting than the parent lines also in the greenhouse, particularly the hybrids between *L. sativa* and *L. serriola*, but the superiority is much less consistent than in the field.

Possible reasons for the better seed setting of the F_1-hybrids will be discussed later. The data in Table 5 show that there is no corresponding improvement in pollen fertility and that the phenomenon cannot be due to differences in flowering time.

2. L. serriola

A. Pure lines

a. *Origin and characteristics*

Both the typical lobed form and the non-lobed form, f. *integrifolia* BOGENHARD, are represented in the material of *L. serriola*, which was obtained partly from wild growing populations and partly from botanic gardens. The following nine lines were used:

Sc.L	from wild population,	Lund, Sweden	Lobed
Sc.S.Afr.	„ „ „	South Africa	„
Sc.R	„ „ „	Romford, England	„
Sc.J	„ Botanic Garden,	Jena, Germany	„
Sc.Sac.	„ wild population,	Portugal	„
Sc.H	„ „ „	Harwich, England	Non-lobed
Sc.Fr.	„ „ „	Baileul, France	„
Sc.aug.All.	„ „ „	Aosta, Italy	„
Sc.int.Sac.	„ „ „	Portugal	„

The line Sc. aug. All. represents a non-lobed, non-prickly form to which ALLIONI (1785) gave the rank of species under the name *L. augustana* (cf. LINDQVIST, 1960 c).

Differences were found between different lines originating from the same population, particularly with regard to flowering time, but the individual lines were true-breeding from the beginning. The populations were evidently mixtures of different homozygous lines, which is in accordance with STEBBINS' conception of the breeding structure of self-fertilizing plants (STEBBINS, 1957).

TABLE 5. *Seed setting in F_1-hybrids and parent lines grown on the field.*

Hybrids	Seed setting			Diff. F_1-best parent	Number of days to flowering			Pollen fertility		
	F_1	Parents			F_1	Parents		F_1	Parents	
L. sativa × *sativa*										
Asp. × K. driv	77.0	—	45.8	—	138	159	137	78.6	93.9	96.5
LC × Altaica	80.1	43.2	41.2	+ 36.9	136	133	141	95.4	96.0	81.9
Altaica × SL	63.5	41.2	46.2	+ 17.3	131	141	130	55.5	81.9	94.2
K. driv × Altaica	82.1	45.8	41.2	+ 36.3	137	137	141	98.8	96.5	81.9
K. driv × SL	74.5	45.8	46.2	+ 28.3	127	137	130	52.6	96.5	94.2
L. sativa × *serriola*										
K. driv × Sc. J	76.7	45.8	45.9	+ 30.8	134	137	151	60.7	96.5	88.4
Altaica × Sc. J	72.4	41.2	45.9	+ 26.5	139	141	151	46.9	81.9	88.4
SL × Sc. J	55.7	46.2	45.9	+ 9.8	134	130	151	46.0	94.2	88.4

b. *Cytology*

Chromosome morphology: — Somatic chromosomes were studied in all the lines except Sc. R. No differences were observed between the different lines except with regard to size of satellites. The chromosomes of the line Sc. L are illustrated in Fig. 4. Measurements were made on the chromosomes in 10 cells of the line Sc. H. The results are presented diagrammatically in Figs. 9 and 14 in the same way as described above for *L. sativa* (page 82). A comparison between the diagrams for the two species shows that the chromosomes of *L. serriola* and *L. sativa* are extremely similar.

As in *L. sativa* variation was found between different lines with regard to the size of satellites (Figs. 25—27). The two SAT-pairs from eight lines were classified as follows:

Line	SAT I	SAT II	Fig.
Sc.H	s	s	26
Sc.Fr.	s	s	—
Sc.aug.All.	m	s	27
Sc.int.Sac.	l	m	—
Sc.L	m	l	25
Sc.S.Afr.	m	m	—
Sc.J	m	m	—
Sc.Sac.	l	m	—

Observations were made on some F_2-seedlings of the cross Sc.H × Sc.L in order to study the inheritance of satellite size. The seeds germinated badly and the slides were of rather poor quality. The satellites could therefore be classified with certainty in four seedlings only with the following results:

Plant no.	SAT I	SAT II
1	ms	ll
2	ms	ll
3	ss	ss
4	mm	ls

The satellites apparently retain their characteristic size and F_2-plants are found, in which the two members of a SAT-pair originate from different parent lines. The SAT-chromosomes of plant no. 4 are illustrated in Fig. 32.

Observations which have been made on a small number of progenies of population plants, indicate that most, perhaps all, plants had the same satellite size in the populations from which these plants originated. Thus, twelve progenies from a population at Malmö, Sweden, all had satellites of the type mm ll, and the same was true for ten progenies from a population at Hälsingborg, Sweden. It is interesting that these two populations, which are separated by a distance of only about 30 miles, are characterized by satellites of the same type as the line Sc.L, which originates from the town of Lund, situated between Malmö and Hälsingborg. Satellites of the type ss ss are presumably typical for a population at Harwich, England. Two progenies have been studied from this population, and they both agreed with the line Sc.H, which originates from a plant, which grew on the same locality 20 years earlier.

Meiosis: — Meiosis was studied in the three lines Sc.R, Sc.H and Sc.S.Afr. and was found to be perfectly normal, as shown by the data in Table 6.

c. *Fertility*

Data on pollen fertility and seed setting are found in Tables 7 and 8 respectively. As in *L. sativa*, plants with a pollen fertility lower than 90 % occurred, particularly among plants grown in the field.

L. serriola is fairly resistent to attacks by fungi, and seed setting out of doors is therefore much more reliable than in cultivated lettuce. This applies also to the other two wild species, *L. saligna* and *L. virosa*.

TABLE 6. *Meiosis in L. serriola.*

Lines or hybrids	No. of plants	I M						I A + I T			II M			II A + II T			% of good pollen	% seed setting
		Pairing				Ring-rod II												
		No. of PMC with		Number of cells	% PMC with univ.	Average number of ring biv. per cell	Number of cells	% cells with bridges	% cells with lagg. chrom.	Number of cells	% cells with bridges	% cells with lagg. chrom.	Number of cells	% cells with bridges	% cells with lagg. chrom.	Number of cells		
		9 II	8 II 2 I															
Lines																		
Sc. R	1 (F)	52		52	0.0	6.9	52	—	—	—	—	—	—	—	—	—	78.1	96.7
Sc. H	1 (G)	71	1	72	1.4	7.7	63	0	0	27	0	0	23	0	0	9	88.6	—
Sc. S. Afr.	2 (G)	35		35	0.0	8.0	35	0	0	15	0	0	5	0	0	4	98.4	—
Hybrids																		
Sc. H × Sc. L	1 (G)	66		66	0.0	7.0	61	0	0	33	0	0	70	0	0	62	88.9	74.5
Sc. S. Afr. × Sc. Fr	2 (G)	78	2	80	2.5	7.8	63	0	0	57	0	0	35	7.7	0	52	97.6	—

(G) = Grown in the greenhouse (F) = Grown on the field

TABLE 7. *Pollen fertility in L. serriola.*

Lines or hybrids	Greenhouse					Field				
	% good pollen					% good pollen				
	100	90	80	70	Average	100	90	80	70	Average
Lines										
Sc. L	8				98.5	4	1			96.8
Sc. H	17	2			95.7	5				96.8
Sc. S. Afr.	10				97.6	4	1			95.6
Sc. R	9				96.6	5				96.0
Sc. J	11	—	1		94.8	4	1	1		91.3
Sc. Fr.	5	1			95.1	3				95.2
Sc. aug. All.	6				97.4					
Hybrids										
Sc. H × Sc. L	1	3	1		86.7	1				90.6
Sc. H × Sc. R					—	5				98.8
Sc. R × Sc. L					—	1				90.0
Sc. S. Afr. × Sc. R					—	2	2			93.9
Sc. S. Afr. × Sc. Fr.	5				97.8					—

Therefore seed fertility data for plants grown in the field have been used for these three species in addition to data from greenhouse plants.

Seed setting was on the whole better in *L. serriola* than in *L. sativa*, but the line Sc.S.Afr. had a remarkably low and variable seed fertility. The poor seed setting in the field plants of Sc.J was due to very unfavourable weather.

B. Hybrids within L. serriola

All crosses attempted between different lines of *L. serriola* were successful and the F_1-hybrids showed normal development. Meiosis was studied in two combinations. No abnormalities were observed apart from a few PMC with univalents and some cases of difficult separations in II A cells of the hybrid Sc.S.Afr.×Sc.Fr. (Table 6). The pollen fertility (Table 7) was similar to that of the parent lines in all hybrids except Sc.H×Sc.L, which had a rather low percentage of good pollen. Data on seed setting are available only for three plants of the hybrid Sc.H×Sc.L (Table 8). They indicate that the seed fertility is equal to that of the parent lines.

TABLE 8. *Seed setting in L. serriola.*

Lines or hybrids	Greenhouse									Field							
	% seed setting									% seed setting							
	100	90	80	70	60	50	40	30	Average	100	90	80	70	60	50	40	Average
Lines																	
Sc. L	2	6	1						86.8				1	1			72.6
Sc. H	3	5	2						87.6	2							94.3
Sc. S. Afr.		1	—	1	2	2	1		55.3				1				79.6
Sc. R		1	2						77.7			2					87.3
Sc. J	2	1	3						83.0						1	2	45.9
Sc. Fr.	1	2							89.7								—
Hybrid																	
Sc. H × Sc. L	2	—	1						89.0								

3. L. saligna

A. Pure lines

a. *Origin and characteristics*

Three lines of *L. saligna* have been used in this study, namely Sal.C, from the botanic garden of Coimbra, Portugal, Sal.R from the botanic garden of Rome and Sal.S, originating from a wild population at Seewinkel, Austria. Young plants of the three lines are almost indistinguishable, but adult plants of Sal.C and Sal.S have linear, almost entire stem leaves, whereas Sal.R has the broader, clearly lobed stem leaves typical of var. *runcinata* NOB.

b. *Cytology*

Chromosome morphology: — Somatic chromosomes were studied in the two lines Sal.C and Sal.R. Measurements were made in 10 cells of the latter line and the results are presented diagrammatically in Figs. 10 and 15 as for the other species. The chromosomes of this line are illustrated in Fig. 5.

The chromosome complement of *L. saligna* is on the whole rather similar to that of *L. sativa* and *L. serriola* and as in these two species there are two SAT-pairs. There are, however, certain differences. Thus the smaller SAT-pair is in *L. saligna* shorter in relation to the other chromosomes, and the chromosome set can be divided into three longer and six shorter pairs, whereas in *L. sativa* and *L. serriola* there is a

TABLE 9. *Meiosis in L. saligna and L. virosa.*

Lines or hybrids	No. of plants	I M						I A+I T			II M			II A+II T				% good pollen
		Pairing				Ring-rod II												
		No. of PMC with		Number of cells	% PMC with univ.	Average number of ring biv. per cell	Number of cells	% cells with bridges	% cells with lagg. chrom.	Number of cells	% cells with bridges	% cells with lagg. chrom.	Number of cells	% cells with bridges	% cells with lagg. chrom.	Number of cells		
		9 II	8 II 2 I															
L. saligna																		
Sal. R	1 (G)	87	2	89	2.2	6.3	64	0	0	8	0	0	13	0	0	4		97.6
Sal. C	1 (G)	109	2	111	1.8	5.5	78	8.3	0	12	0	0	9	0	0	5		99.5
Sal. R × Sal. C	1 (G)	58		58	0.0	6.6	58	0	0	68	5.6	0	18	0	0	20		—
L. virosa																		
Vir. L	1 (G)	49		49	0.0	7.5	41	—	—	—	14.3	0	7	—	—	—		—
Vir. J	1 (G)	124	3	127	2.4	7.6	74	4.8	0	21	0	0	17	0	0	33		99.6

(G)=Grown in the greenhouse

TABLE 10. *Pollen fertility in L. saligna and L. virosa.*

Lines or hybrids	Greenhouse		Field					
	% good pollen		% good pollen					
	100	90 Average	100	90	80	70	60	50 Average
L. saligna								
Sal. R	22	97.6	3	1				93.5
Sal. C	12	97.9	4					98.2
Sal. R × Sal. C	6	99.6						—
L. virosa								
Vir. L	3	97.8	1					99.0
Vir. J	2	99.4	3					97.3
Vir. K	1	97.1	1					97.1
Vir. C		—	2					93.9
Vir. C × Vir. K		—				1	2	56.4

more continuous variation in the length of the chromosomes. No differences between the two lines were detected, except with regard to size of the satellites. In Sal.C these were of the type ll ss, in Sal.R, ll mm, the first letters referring to the satellites of the shorter pair (Figs. 30 and 31).

Meiosis: — Meiosis was studied in Sal.C and Sal.R and found to be normal, as shown by the data in Table 9. The chiasma frequency, measured by the average number of ring bivalents per cell, appeared to be lower than in *L. sativa* and *L. serriola*, but bivalent pairing was almost perfect and hardly any abnormalities were found in later stages of meiosis.

c. *Fertility*

Data in Tables 10 and 11 show that pollen fertility and seed setting were good in both lines.

B. Hybrids within L. saligna

Only one hybrid within *L. saligna* has been studied, namely Sal.R × × Sal.C. This showed absolutely normal vegetative development. Meiosis was normal, except for a single bridge persisting until II M (Table 9). Pollen and seed fertility were good as shown by the data in Tables 10 and 11.

TABLE 11. *Seed setting in L. saligna and L. virosa.*

Lines or hybrids	Greenhouse						Field				
	% seed setting						% seed setting				
	100	90	80	70	60	Average	100	90	80	70	Average
L. saligna											
Sal. R	4	4				90.3		1			86.2
Sal. C	6	1	—	1		91.9	1				95.5
Sal. R × Sal. C	3					96.7					—
L. virosa											
Vir. L	3					93.5	3	1			95.0
Vir. K						—	1	2	1		85.2
Vir. C						—		1			82.1
Vir. T		3	1			83.7					—

4. L. virosa

A. Pure lines

a. *Origin and characteristics*

The material of *L. virosa* consisted of six lines with the following names and origins:

Vir.K	Royal Botanic Gardens, Kew, England
Vir.L	Botanic Garden, Lund, Sweden
Vir.J	„ „ Jena, Germany
Vir.C	Wild population, Norfolk, England
Vir.T	„ „ „ „
Vir.S	„ „ „ „

The lines showed considerable differences in morphological characters, such as anthocyanin colour, leaf form and spination. The last three lines appeared to be identical in every respect. They originate from three different Norfolk populations, separated by distances of 20 to 60 miles.

In contrast to the other three species of the *serriola* group, *L. virosa* is biennial, although some plants can be induced to flower the first year by early sowing.

b. *Cytology*

Chromosome morphology: — Clear differences in chromosome morphology were found between *L. virosa* and the other members of the *serriola* group. This is shown by the illustration of the chromosomes of

the line Vir.L in Fig. 6 and by the diagrams in Figs. 11 and 16. There is only one pair of chromosomes with satellites. This corresponds to the shorter SAT-pair in the other three species. Two of the shorter chromosomes in the complement of *L. virosa* have one very short arm. No chromosomes with a corresponding position of the centromere were seen in *L. sativa*, *L. serriola* or *L. saligna*.

Three lines of *L. virosa* were examined, namely Vir.L, Vir.J and Vir.T, but no differences were observed between the chromosomes of the three lines, which agreed also in the size of the satellites (Figs. 28 and 29).

Meiosis: — Meiosis was studied in the two lines Vir.L and Vir.J and the data in Table 9 show that very few irregularities were observed.

c. *Fertility*

Pollen fertility and seed setting were normal in all the lines (Tables 10 and 11).

B. Hybrids within L. virosa

Only one hybrid was produced between lines of *L. virosa*, namely Vir.C×Vir.K. The vegetative development was normal. Meiosis was studied on slides made after imbedding in parafin wax, sectioning and staining in crystal violet. These slides were inferior to those prepared by the squash method and were not suitable for detailed analysis. No serious disturbances were observed. Pollen fertility was, however, low in two plants grown on the field (Table 10), whether for genetic reasons or because of unfavourable weather, cannot be decided. No data are available for seed setting.

5. Interspecific hybrids

A. L. sativa×serriola

a. *Description of hybrids*

Crosses between *L. sativa* and *L. serriola* succeed easily in both directions. Twenty six different combinations were successfully attempted, involving 16 lines of *L. sativa* (12 cultivated+4 primitive) and 5 lines of *L. serriola*. As in the case of the hybrids within *L. sativa*, there was no general striking heterosis effect, although some combinations exceeded the best of the parents in some characters.

b. *Cytology*

Chromosome morphology: — Observations on satellite size were made

in six F_2-plants of the cross Altaica×Sc.H. The satellites of Altaica are of the type mm ss and those of Sc.H ss ss, *i.e.* the parents differ in the size of the satellites on the shorter of the two SAT-pairs. Of the six F_2-plants two agreed with each parent and two were recombinations of the type ms ss, illustrated in Fig. 33. This confirms the evidence from F_2 of the cross Sc.H×Sc.L, that satellite size is a constant chromosomal character.

Meiosis: — Meiosis was on the whole regular in hybrids between *L. sativa* and *L. serriola.* Data for 11 combinations are presented in Table 12. Irregularities were less frequent than in pure lines and hybrids within *L. sativa.*

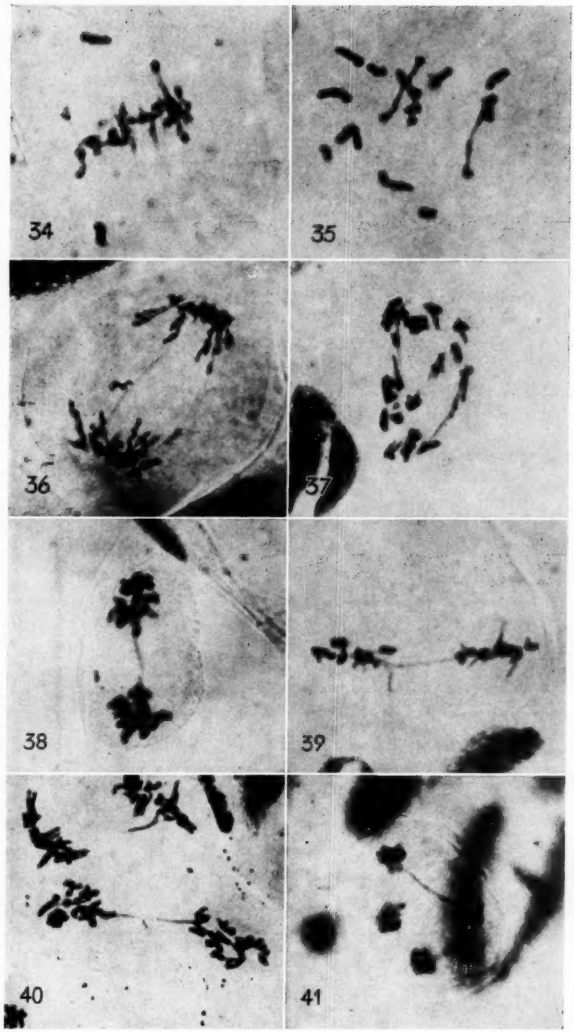
c. *Fertility*

Pollen fertility: — The data in Table 13 show that partial pollen sterility is found in many hybrids between lines of *L. sativa* and *L. serriola.* Different lines of *L. serriola* have given different results in crosses with cultivated forms of *L. sativa.* The two lines Sc.L and Sc.R gave hybrids with normal pollen fertility, whereas partial pollen sterility was found in hybrids with Sc.S.Afr., Sc.J and Sc.H. The data in Table 13 concerning the lines Sc.H are very scant, but they are supported by observations on three other hybrids, namely K.driv×Sc.H, Merv.×Sc.H and R.rouge×Sc.H, which have been recorded as partly pollen sterile, although no exact data are available.

With the exception of three hybrids with Altaica, all F_1-hybrids between *L. serriola* and primitive forms of *L. sativa* were found to be partly pollen sterile. It should be recalled that the primitive forms SL and SP gave partial pollen sterility also in hybrids with cultivated forms.

If the results of hybrids within *L. sativa* and hybrids between *L. sativa* and *L. serriola* are considered together, it is found that seven lines have consistently given hybrids with partial pollen sterility, namely Asp., SL, SB, SP, Sc.H, Sc.S.Afr. and Sc.J. Exceptions are hybrids between some of these lines and also the two hybrids Altaica×Sc.H and Altaica× ×Sc.S.Afr.

A correlation has been found between the partial pollen sterility in F_1 and disturbed segregation ratios in F_2 for the gene pair *G—g*, for green versus yellow leaf colour. A very serious deficiency in the recessive class is found in many crosses (LINDQVIST, 1960 b) and it is significant that such discrepancies always occur in crosses, in which one of the seven above mentioned lines is taking part, and only in such crosses. For com-



Figs. 34—41. Meiosis in *L. sativa*. — Fig. 34. Tezier cos; I M with two univalents. Fig. 35. F_1 SB×SL; I M with 12 univalents. Fig. 36. Tezier cos; I A with bridge and fragment. Fig. 37. F_1 Altaica×SL; difficult separation at I A. Fig. 38. F_1 Altaica×SL; I T with bridge and fragment. Fig. 39. Tezier cos; II M with bridge persisting from I A. Fig. 40. SP; ditto. Fig. 41. F_1 Altaica×SL; II A with bridge persisting from I A.

TABLE 12. *Meiosis in hybrids between L. sativa and L. serriola.*

Hybrids	No. of plants	I M						I A+I T			II M			II A+II T			% good pollen	% seed setting
		Pairing				Ring-rod II												
		No. of PMC with		Number of cells	% PMC with univ.	Average number of ring biv. per cell	Number of cells	% cells with bridges	% cells with lagg. chrom.	Number of cells	% cells with bridges	% cells with lagg. chrom.	Number of cells	% cells with bridges	% cells with lagg. chrom.	Number of cells		
		9 II	8 II 2 I															
K. driv × Sc. J	1 (F)	125	1	126	0.8	7.5	125	—	—	15	—	—	63	—	—	15	63.2	74.0
Maik. × Sc. S. Afr.	1 (G)	72	2	74	2.7	7.5	72	—	—	14	—	—	7	—	—	—	51.7	71.3
Berl. × Sc. L	1 (G)	99	3	102	2.9	6.5	96	—	—	38	—	—	47	—	—	34	78.8	—
Paris × Sc. J	1 (G)	43		43	0.0	7.3	39	—	8.3	12	—	2.9	34	—	15.4	13	—	—
R. rouge × Sc. S. Afr.	1 (G)	100	2	102	2.0	7.5	95	2.7	—	37	—	—	—	—	—	—	—	—
Altaica × Sc. J	1 (F)	105		105	0.0	7.0	105	—	—	26	—	—	46	—	—	21	46.2	69.6
Altaica × Sc. H	1 (G)	32		32	0.0	—	—	—	—	11	—	3.2	62	—	—	29	96.6	—
LC × Sc. H	1 (G)	22	1	23	4.3	—	—	—	—	12	—	—	2	—	—	4	64.1	92.9
SL × Sc. J	1 (F)	191	3	194	1.5	7.5	75	—	—	15	—	—	9	—	3.1	64	—	—
SL × Sc. L	1 (G)	28		28	0.0	7.3	26	—	—	3	—	—	5	—	—	2	46.3	—
Sc. S. Afr. × SM	1 (G)	19		19	0.0	7.8	16	—	—	16	10.0	10.0	10	—	—	39	78.4	—

(G)=Grown in the greenhouse (F)=Grown in the field

TABLE 13. *Pollen fertility in hybrids L. sativa×serriola.*

Hybrids	Greenhouse								Field							
	% good pollen								% good pollen							
	100	90	80	70	60	50	40	Average	100	90	80	70	60	50	40	30	Average
Berl. × Sc. L	7	—	1					95.2	5	1							94.2
Paris × Sc. L								—	1								98.0
Sc. L × T + g								—	1								97.1
Berl. × Sc. R	2							96.2	2								94.6
Berl. × Sc. S. Afr.			3					74.4				2	2				62.2
Maik. × Sc. S. Afr.					2	1		50.6						1	1		38.2
R. rouge × Sc. S. Afr.		1	5					76.9					3	1	3		42.9
Oakl. × Sc. S. Afr.								—					3	2			50.2
K. driv × Sc. J								—				2	1				60.7
Paris × Sc. J			2	4				69.4									—
Sc. H × T + g								—			1						72.1
Berl. × Sc. H'								—				1					67.5
Altaica × Sc. H	2	3	1					87.2	1	1							89.6
Sc. L × Altaica								—	2	1							94.5
Sc. S. Afr. × Altaica								—	2	1							90.0
Altaica × Sc. J								—					1	1	1		46.9
SL × Sc. L				1	2	1		55.1				1	2	1	2		49.6
SL × Sc. J								—						3			46.0
Sc. L × SP								—					3				53.7
Sc. S. Afr. × SM			2	—	1			68.2									—
LC × Sc. H				1	1			58.8									—

plete data the reader is referred to the paper mentioned above. The results below exemplify the different segregation ratios obtained in progenies of F_1-hybrids with normal pollen fertility and of hybrids with partial pollen sterility. The figures for pollen fertility are averages of all available data, *i.e.* from greenhouse and field plants.

Cross	Pollen fertility in F_1	Segregation in F_2 G	g	Total	χ^2 for 3:1
Berl.×Sc.L	94.8	241	70	311	1.030
Berl.×Sc.R	95.4	190	52	242	1.592
Berl.×Sc.S.Afr.	67.4	340	42	382	39.962***
Berl.×Sc.H	67.5	783	84	867	107.839***

This correlation between pollen fertility and segregation ratios is a valuable confirmation that the partial pollen sterility of certain hybrids is due to causes other than environmental factors. The data in Tables

TABLE 14. *Pollen fertility in F_2 of crosses between L. sativa and L. serriola.*

Hybrids	Generation	% good pollen								n
		100	90	80	70	60	50	40	30	
Berl. × Sc. L	F_1 (G)	7	—	1						7
	F_1 (F)	5	1							6
	F_2 (F)	136	2	1						139
R. rouge × Sc. S. Afr.	F_1 (G)		1	5						6
	F_1 (F)					3	1	3		7
	F_2 (F)	62	22	14	11	—	1			110
Maik. × Sc. S. Afr.	F_1 (G)					2	1			3
	F_1 (F)							1		1
	F_2 (F)	16	6	8	8	1	3			42

(G) = Grown in the greenhouse (F) = Grown in the field

2 and 13 are by themselves not entirely convincing on this point, because of the occurrence of partly pollen sterile plants also among the parent lines and because of the small number of plants studied of each line and hybrid.

In hybrids between the line Asp. and the cultivated varieties Berl. and Oakl., greenhouse plants had normal pollen fertility, but field plants were partly pollen sterile. The leaf colour genes *G* and *g* segregated in F_2 of the cross Berl. × Asp. The data below show, that the difference in pollen fertility caused by different environments, had no effect on the segregation ratios.

F_1-plants from	Pollen fertility in F_1	F_2-segregation G	g
greenhouse	97.9	230	54
field	79.3	241	56

χ^2 for heterogeneity 0.002

These facts suggest that the percentage of functionable pollen was the same in the F_1-plants grown in the greenhouse and in those grown in the field, in spite of the difference in the percentage of morphologically good pollen.

Pollen fertility was studied in F_2-generations of three crosses between *L. sativa* and *L. serriola*, namely Berl. × Sc.L, which had normal pollen fertility in F_1, and R.rouge × Sc.S.Afr. and Maik. × Sc.S.Afr., which both showed partial pollen sterility in F_1. The results are recorded in Table 14. In F_2 of Berl. × Sc.L practically all the plants had normal pollen

TABLE 15. *Seed setting in hybrids L. sativa×serriola.*

Hybrids	Greenhouse					Field							
	% seed setting					% seed setting							
	100	90	80	70	Average	100	90	80	70	60	50	40	Average
Berl. × Sc. L		1			89.0	3							98.6
Paris × Sc. L					—	1							94.5
Berl. × Sc. R	1	—	1		87.2				1				66.0
Sc. L × T + g					—	2	2						90.2
Berl. × Sc. S. Afr.	2	1			92.0	3							93.0
Maik. × Sc. S. Afr.			3		71.7		2	3	1				76.0
R. rouge × Sc. S. Afr.	2	3	1		86.4								—
Oakl. × Sc. S. Afr.					—		6						85.7
K. driv × Sc. J					—		1	1	1				76.7
Paris × Sc. J	3				97.0								—
Altaica × Sc. H	2				97.4								—
Sc. L × Altaica					—	3							94.0
Altaica × Sc. J					—			2	1				72.4
SL × Sc. L	3				94.3								—
SL × Sc. J					—				1	1	1		55.7
Sc. L × SP					—	3							94.4
LC × Sc. H	1	1			90.0								—

fertility, but in the other two crosses segregation took place into plants with normal fertility and partly sterile plants. The segregations were continuous in both crosses and it is not possible to draw any conclusions about the mode of inheritance.

A comparison of the data in Tables 1 and 2 and 12 and 13 respectively, makes it clear that the partial pollen sterility in F_1-hybrids is not normally connected with any meiotic irregularities. On the contrary, the F_1-hybrids have, as a rule, better pairing and less disturbances at I A than the parent lines and this applies also to hybrids with reduced pollen fertility.

Seed setting: — Data on seed setting in greenhouse and field plants of hybrids between *L. sativa* and *L. serriola* are presented in Table 15. The female fertility is good in most hybrids, often better than in the parent lines (*cf.* page 94). Seed setting is not strongly correlated with pollen fertility, some hybrids with partial pollen sterility having a somewhat reduced seed setting (*e.g.* Maik.×Sc.S.Afr., K.driv×Sc.J, Altaica×Sc.J and SL×Sc.J), others having very good seed setting (*e.g.* Berl.×Sc.S.Afr., Paris×Sc.J and Sc.L×SP).

B. L. sativa×saligna

a. *Description of hybrids*

Numerous attempts have been made to cross *L. sativa* and *L. saligna* with *L. sativa* as female parent, but they have all failed. Two cultivated varieties, Berl. and Oakl., and three primitive forms, Altaica, SP and SL, of *L. sativa* have been used as female partners, and the two *L. saligna* lines Sal.R and Sal.C as pollinators. In all 139 flower heads were pollinated, but only shrivelled, undeveloped seeds were obtained which failed to germinate.

When *L. saligna* was used as the female parent, hybrid seeds were usually obtained without difficulty. These differed in appearance from seeds produced by the mother plant after selfing. Hybrid seeds were smaller, although usually quite firm and well filled, and the seed coat varied in colour from dark brown to very pale, almost white. Seeds suspected to be the result of selfing were sown separately. Examination of the seedlings showed that separation of the two kinds of seeds by their appearance was usually successful, but that a certain amount of overlapping existed. The difference in appearance between selfed and hybrid seeds is illustrated by Figs. 42—45.

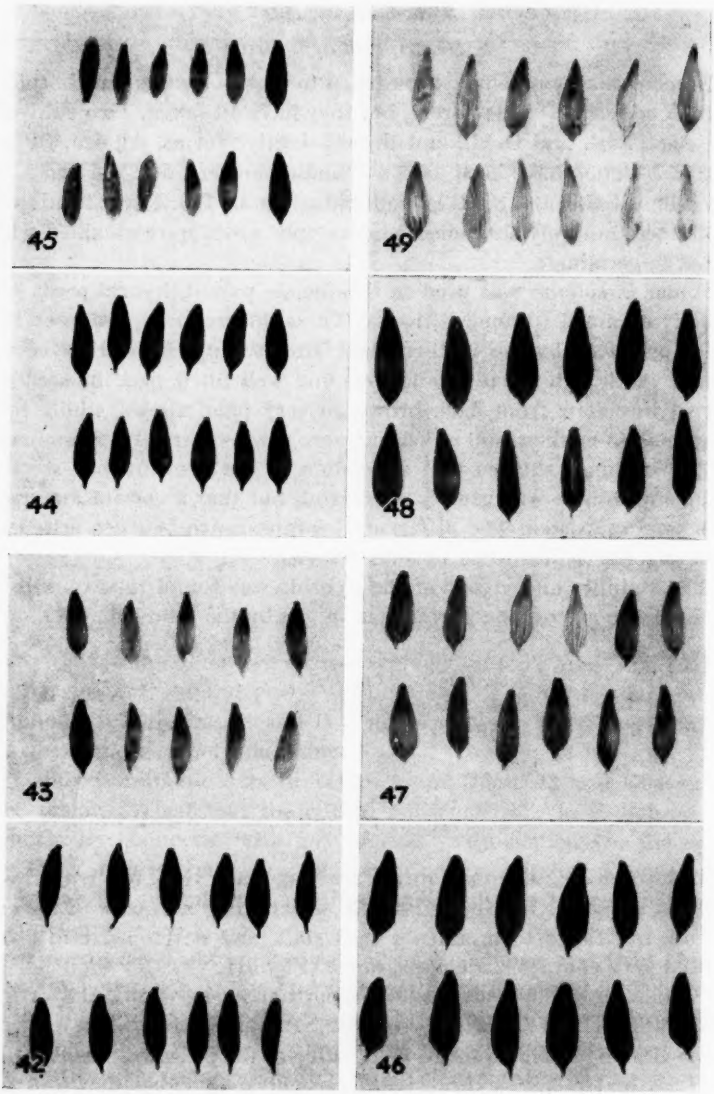
The viability and vigour of the hybrids was found to vary with the lines chosen as crossing partners as shown by the following data.

Female parent	Male parent	Hybrids
Sal.R	Berl., Asp., G.W.Cos, Oakl., Maik., Altaica, LC, SM, SB	Died in seedling stage
Sal.R	SL, SP	Dwarfs
Sal.S	SL	Dwarfs
Sal.C	SM, SL, SP	Dwarfs
Sal.C	K.driv, Asp., Oakl., Berl., R.rouge, Altaica, LC, SB, ST	Normal vigour

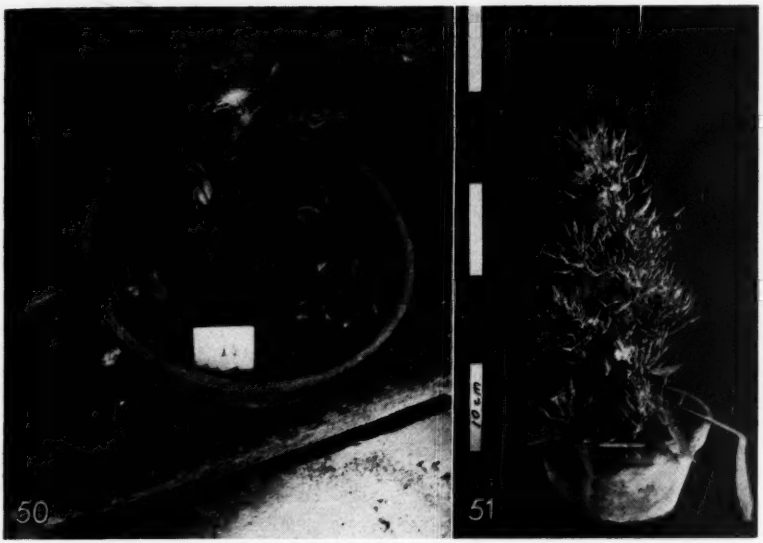
Hybrids with Sal.R died in the seedling stage (Fig. 50) with the exception of Sal.R×SL and Sal.R×SP which grew normally in the early stages, but failed to produce a seed stalk and developed into dwarfs with a very characteristic appearance (Fig. 51).

Similar dwarf hybrids resulted from crosses between Sal.S or Sal.C and the primitive *L. sativa* forms SL, SP and SM, but when Sal.C was crossed with cultivated forms of *L. sativa* or the primitive forms Altaica, LC, SB and ST, F_1-hybrids with normal stem elongation and good vigour were obtained (Fig. 52).

However, also these hybrids showed certain morphological peculiarities. Leaf growth was often abnormal, resulting in forking and in aereas



Figs. 42—49. Defective seeds from interspecific matings. — Fig. 42. Seeds of *L. saligna*, line Sal.R, after selfing. Fig. 43. Ditto after pollinating with *L. sativa*. Fig. 44. Seeds of *L. saligna*, line Sal.C, after selfing. Fig. 45. Ditto after pollinating with *L. sativa*. Fig. 46. Seeds of *L. sativa*, line Altaica, after selfing. Fig. 47. Ditto after pollinating with *L. virosa*. Fig. 48. Seeds of *L. sativa*, line SL, after selfing. Fig. 49. Ditto after pollinating with *L. virosa*.



Figs. 50—51. F_1-hybrids *L. saligna*×*sativa*. — Fig. 50. Sal.R×Berl.; the big plants is an accidental self. Fig. 51. Sal.C×SM; dwarf.

Fig. 52. F_1-hybrid *L. saligna*×*sativa*, Sal.C×Altaica.

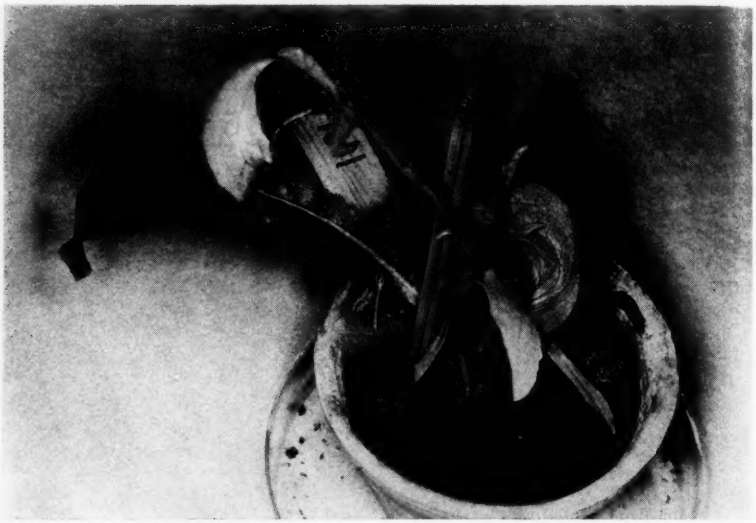


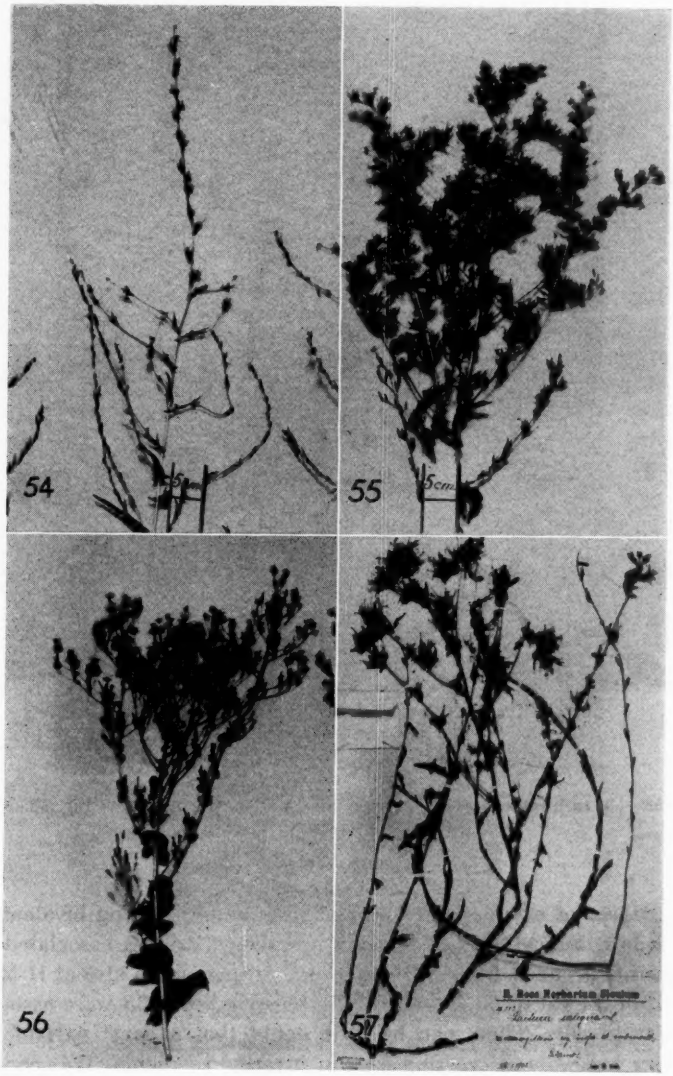
Fig. 53. F_1-hybrid *L. saligna*×*sativa*, Sal.C×SB.

of necrotic tissue (Fig. 53). This abnormal growth was reminiscent of that resulting from colchicine treatment of young seedlings. The stem was more or less angular, which is surprising, as all the species of the *serriola* group have round stems. The internodes were of unequal length, sometimes very short. The inflorescence had a very characteristic appearance, being a compromise between the *L. saligna* type with strong apical dominance and very short peduncles and the much more spreading *L. sativa* inflorescence with longer peduncles (Figs. 54—56).

Cytological evidence will be presented later to show that hybrids between *L. sativa* and *L. saligna* often are chimaeras of diploid and tetraploid tissue. Morphological evidence of this is provided by the abnormal leaf growth mentioned above and by the fact that whole twigs are occasionally found with buds and flower heads which are bigger than normal (Figs. 58 and 59). Such flower heads have much larger pollen grains than other flower heads on the same plant.

b. *Cytology*

Data on meiosis in hybrids between *L. saligna* and *L. sativa* are found in Table 16 and illustrations are found in Figs. 60—63. There is a rather



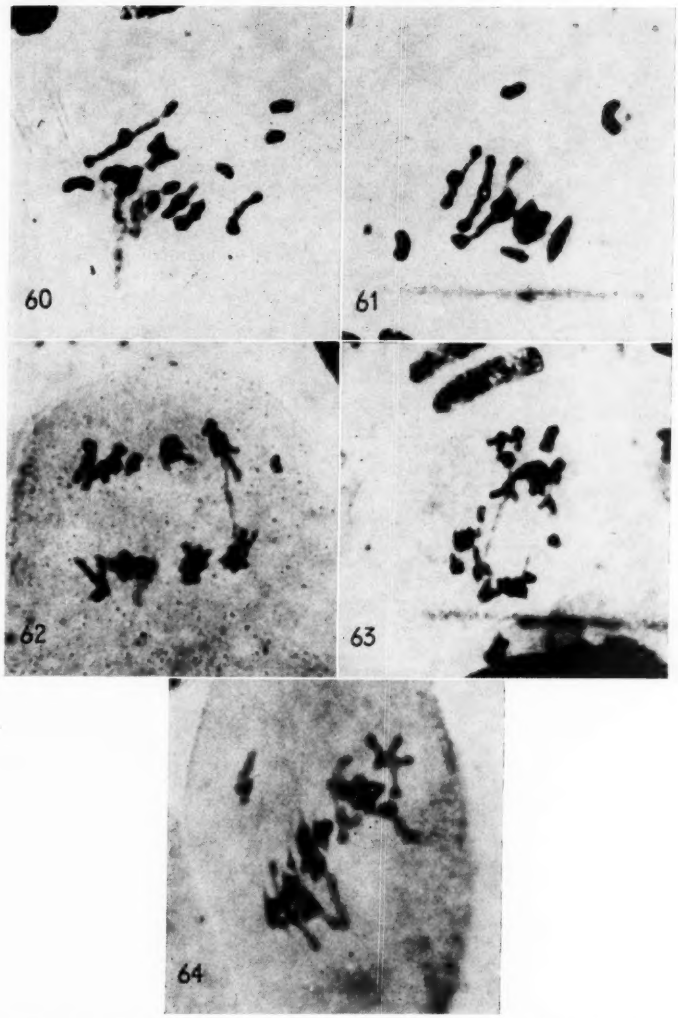
Figs. 54—57. Specimens of *L. sativa*, *L. saligna*, a hybrid and a suspected hybrid. — Fig. 54. Inflorescence of *L. saligna*, line Sal.C. Fig. 55. Inflorescence of F_1 *L. saligna*× ×*sativa*, Sal.C×R.rouge. Fig. 56. Inflorescence of *L. sativa*, line R.rouge. Fig. 57. Herbarium specimen of suspected hybrid *L. saligna*×*sativa* or *L. saligna*×*serriola*.

Figs. 58—59. Inflorescences of F_1 *L. saligna*×*sativa*, Sal.C×SB. — Fig. 58. Normal twig. Fig. 59. Tetraploid twig.

high degree of asyndesis at I M and the number of ring bivalents per cell is low, indicating low chiasma frequency. Bridges associated with fragments occur at I A and II A. Bridges are common also at II M.

Out of a total of 59 I A-bridges in these hybrids, 35 were associated with fragments. There can be little doubt that at least part of these bridges were due to crossing over in inverted segments. This confirms the existence of structural differences between the chromosomes of *L. sativa* and *L. saligna*, which was suggested by the morphology of the somatic chromosomes.

In two buds of the hybrid Sal.C×SL all PMC were found to be tetra-



Figs. 60—64. Meiosis in *L. saligna*×*sativa*. — Fig. 60. Sal.C×K.driv; I M with 4 univalents. Fig. 61. Sal.C×LC; I M with 4 univalents. Fig. 62. Sal.C×K.driv; I A with bridge and fragment. Fig. 63. Sal.C×LC; I A with bridge and fragment. Fig. 64. Sal.C×SL; tetraploid PMC at I M.

TABLE 16. *Meiosis in hybrids L. saligna×sativa and L. saligna×serriola.*

Hybrids	No. of plants	I M									I A+I T			II M			II A+II T		
		Pairing							Ring-rod II										
		No. of PMC with					Number of cells	% PMC with univ.	Average number of ring biv. per cell	Number of cells	% cells with bridges	% cells with lagg. chrom.	Number of cells	% cells with bridges	% cells with lagg. chrom.	Number of cells	% cells with bridges	% cells with lagg. chrom.	Number of cells
		9 II	8 II 2 I	7 II 4 I	6 II 6 I	<6 II													
L. saligna × sativa																			
Sal. C × K. driv	1	63	28	5	3		99	36.4	5.3	63	25.6	12.8	39	20.0	20.0	10	7.1	14.3	14
Sal. C × Oakl.	1	21	9	1	1		32	34.4	5.6	17	26.3	21.1	19	26.5	5.9	34	50.0	50.0	2
Sal. C × Asp.	2	92	13	3			108	14.8	6.3	82	31.0	6.9	29	30.3	6.1	33	10.0	0	10
Sal. C × R. rouge	1	24	8	6	2		40	40.0	5.7	23	9.2	23.7	76	3.4	10.3	29	11.1	22.2	18
Sal. C × Altaica	1	70	30	5			105	33.3	5.0	55	13.5	9.5	74	15.2	9.1	33	27.8	2.8	36
Sal. C × LC	1	102	31	9	—	1	143	28.7	4.7	102	24.3	16.2	37	10.4	18.2	77	27.1	22.9	48
Sal. C × SL	1	22	8				30	26.7	5.1	22	11.5	15.4	26	13.9	8.3	36	13.9	13.9	36
Sal. C × SM	1	44	5				49	10.2	6.0	41	20.0	0	5	0	0	7	—	—	—
Sal. R × SL	1	66	27	6	3		102	35.3	5.5	63	25.0	20.8	24	18.2	0	11	33.3	8.3	12
L.saligna×serriola																			
Sal. R × Sc. H	1	66	19	2	1	1	89	25.8	4.5	66	15.4	20.5	39	28.6	5.7	35	31.3	12.5	16

TABLE 17. *Pollen fertility in hybrids L. saligna×sativa, L. saligna×serriola, L. sativa×virosa and L. serriola×virosa.*

Hybrids	% good pollen										
	90	80	70	60	50	40	30	20	10	0	Average
L. saligna × sativa											
Sal. C × Asp.						2					34.4
„ × Berl.								1			14.8
„ × Oakl.						2	2	2			25.0
„ × K. driv		1	—	—	2	2	2	1			37.7
„ × R. rouge							4	2			19.3
Sal. C × Altaica						3	1				31.2
„ × LC			1	—	2						51.5
„ × SP							7				25.7
„ × SL		1	—	—	—	—					74.0
„ × SM					1	1	2	1			31.0
„ × SB					1	2	7				28.2
„ × ST		1	2	—	—	3					53.0
Sal. R × SL						1	1				30.8
„ × SP						1	9	4	1		22.5
Sal. Seew. × SL					2	3	3				33.0
L. saligna × serriola											
Sal. C × Sc. H			1								62.7
„ × Sc. L	1										81.7
Sal. R × Sc. H					1	—	1	—	1		26.0
L. sativa × virosa											
Altaica × Vir. K			1	—	1	1	—	1			40.6
„ × Vir. L				1							55.6
„ × F_1 (Vir. K × Vir. C)						2					35.4
F_1 (Vir. K × Vir. C) × Altaica		1									76.8
SL × Vir. L						1					34.9
L. serriola × virosa											
Sc. H × Vir. K		1	—	—	—	—	—	1			46.2
Sc. R × Vir. C								1			13.6
Vir. K × Sc. H			1	—	—	—	—	1	1		27.6
Vir. C × Sc. H						1	—	—	2		15.5
Vir. C × Sc. R							1	—	2		13.3

ploid (Fig. 64), a fact which confirms that doubling of the chromosome number takes place in somatic tissue in hybrids between *L. saligna* and *L. sativa*.

TABLE 18. *Seed setting in hybrids L. saligna×sativa, L. saligna×serriola, L. sativa×virosa and L. serriola×virosa.*

Hybrids	% seed setting							
	50	40	30	20	10	1	0	Average
L. saligna × sativa								
Sal. C × Asp.					1	1		0.5
Sal. C × Berl.						1		< 1.0
Sal. C × Oakl.			1	2				18.3
Sal. C × K. driv			1	1	—	4		6.6
Sal. C × R. rouge					2	1		1.0
Sal. C × Altaica				1				11.3
„ × LC				2	1			12.0
„ × SP						6		0.0
„ × SL						2		0.0
„ × SM					2	2		0.9
„ × SB						21		0.05
„ × ST					1			2.2
Sal. R × SL						2		0.0
„ × SP					3	14		0.3
Sal. Seew. × SL					2	8		0.5
L. serriola × saligna								
Sal. C × Sc. H						1		0.05
„ × Sc. L	1							46.6
Sal. R × Sc. H						3		0.0
L. sativa × virosa								
SL × Vir. L						2		0.0
SL × Vir. K						1		0.1
L. serriola × virosa								
Sc. H × Vir. L					2	2		1.3
Sc. S. Afr. × Vir. K						1		0.3
Vir. K × Sc. H						1		0.06
Vir. C × Sc. H						1		0.3

c. *Fertility*

Data on pollen fertility and seed setting are presented in Tables 17 and 18. The number of plants examined was small and as no marked difference between greenhouse plants and field plants was apparent, they have not been separated in the table. The pollen samples contained a varying proportion of very large pollen grains. There can be

little doubt that these were the result of somatic chromosome doubling and their presence accounts for the surprisingly good pollen fertility found in some cases.

d. *F_2- and F_3-generations*

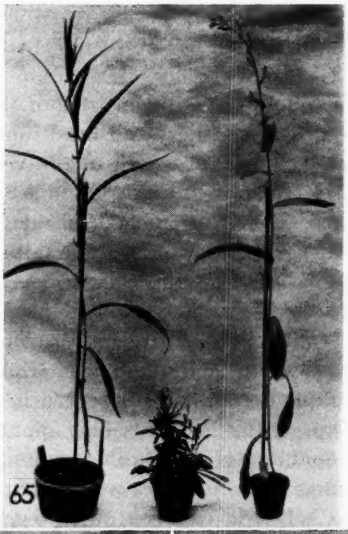
F_2 and F_3 generations were raised of the following three crosses: Sal.C×K.driv, Sal.C×LC and Sal.C×SM.

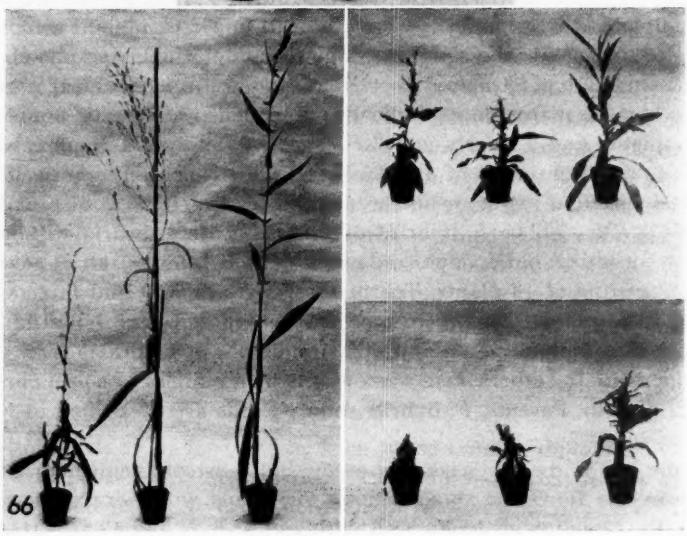
Sal.C×K.driv: — The F_2-generation of this cross consisted of 20 plants. Somatic chromosome numbers were determined in root tips of young seedlings. Not less than 15 of the plants were tetraploid, one was triploid and two were diploid. Some of the plants classified as tetraploid may possibly have been aneuploid. Many of the F_2-seedlings, including several tetraploids, showed the same abnormal leaf growth as the F_1-plants, indicating that they were chimaeras of tissues with different chromosome numbers. Cells with 72 chromosomes were seen in root tips of some tetraploid plants. The tendency to somatic doubling had apparently been inherited by some F_2-plants, tetraploids as well as diploids.

Thirteen of the plants classified as tetraploids survived transplanting to the field. They varied greatly with regard to vigour and morphological characters, but several of the more vigorous plants conformed to a certain type, characterized by long, narrow, pointed, slightly lobed leaves, with rather tough texture. One of the tetraploids, however, differed in many respects, having soft, light coloured, broad leaves, similar to those of cultivated lettuce. The triploid plant had normal vigour and was similar to the tetraploid type described above. Two of the diploids were quite vigorous, early in development, and had long, narrow, pointed leaves, one was a semi-dwarf and the fourth a very weak plant which died at an early stage. The angularity of the stem which was characteristic for the F_1-hybrids, was observed also in many F_2-plants.

No fertility data are available for the F_2-generation, but a fair number of seeds were collected from some tetraploids and one diploid. An F_3-generation was raised, consisting of two families from tetraploid F_2-plants and one from the diploid F_2-plant.

The two progenies of tetraploid F_2-plants consisted of 10 and 6 plants respectively, all tetraploid as shown by chromosome counts. The families segregated in earliness, leaf form and spination. Pollen fertility was good in both families, varying from 80.4 %—97.6 %, under greenhouse conditions. There was a great difference between the two families with regard to seed setting, as shown by the following data:





Figs. 65—66. Parents, F_1 and F_2 of *L. saligna*×*sativa*, Sal.C×SM. — Fig. 65. Left Sal.C, middle F_1, right SM. Fig. 66. F_2-plants.

	Percentage good seeds
Fam. 4191	0.8, 0.9, 1.6, 1.7, 1.8, 1.9
Fam. 4192	10.1, 13.7, 15.0, 29.9, 36.6, 44.0

The progeny of the diploid F_2-plant consisted of 10 plants, all diploid, and uniform with regard to earliness and most morphological characters except leaf form. Some plants were in the vegetative parts very similar to the primitive *L. sativa* lines SL, SP and SM, but the inflorescence resembled that described for the F_1-hybrid. Pollen fertility was good in this family, only two plants out of nine falling below 90 %. The seed setting varied between 2.2 % and 50.8 %.

Chromosome numbers were determined in progenies of six plants from the diploid F_2-family. Five of these gave completely diploid offspring, but the sixth gave one tetraploid, one triploid and 20 diploid plants in F_3, and tetraploid cells were observed in root tips of one of the diploid plants. One of the F_2-plants had apparently inherited the tendency to somatic doubling of the chromosome number.

Sal.C×LC: — Chromosome numbers were determined for 16 plants in F_2 of the cross Sal.C×LC. Seven were found to be tetraploid and nine were diploid. Tetraploid cells were observed in a diploid, and octoploid cells in a tetraploid. Abnormal, weak plants and normal, vigorous plants were found among tetraploids as well as diploids. Abnormal leaf growth was evident in many plants, indicating a tendency to somatic doubling. Many plants had a clearly angular stem. One tetraploid F_2-plant gave an F_3-family consisting of 10 plants, all with 36 chromosomes. They showed variation in vigour and segregated for leaf form and spination.

Sal.C×SM: — The F_1-hybrids of this cross were dwarfs with very poor seed setting, but enough seeds were collected to raise an F_2-generation consisting of 13 plants. Ten of these were diploid and three were triploid. Tetraploid cells were seen in root tips of one of the diploid plants. Two diploid plants and one triploid showed normal vegetative development, the others were weak plants with more or less pronounced dwarf growth. Parents, F_1-hybrid and F_2-plants are illustrated in Figs. 65 and 66.

Some of the dwarfs set a few seeds, but most of them died before reaching the flowering stage. Among the plants with normal development, the two diploids had a seed setting of 11.8 % and 12.8 % respectively, whereas the triploid was completely seed sterile.

The fact that plants with completely normal development appeared in this small F_2-generation, suggests, that the dwarf growth is due to the interaction of certain genes, the number of which is not very large.

Because of the meiotic irregularities in F_1, normal mendelian segregation cannot be expected and therefore it is not possible to draw any conclusions about the number of genes involved.

C. L. serriola×saligna

a. *Description of hybrids*

Five crosses were attempted with *L. serriola* as female partner, namely Sc.H×Sal.R, Sc.aug.All.×Sal.R, Sc.J×Sal.R, Sc.H×Sal.C and Sc.L×Sal.C. In all 95 flower heads were pollinated, but no hybrid seedlings were obtained, only half-developed, shrivelled seeds, which failed to germinate.

Eight crosses were made with *L. saligna* as mother plant, namely Sal.R×Sc.H, Sal.R×Sc.S.Afr., Sal.R×Sc.J, Sal.R×Sc.R, Sal.R×Sc.aug.All., Sal.R×Sc.int.Sac., Sal.C×Sc.H and Sal.C×Sc.L. Hybrid seedlings were obtained in all these combinations except Sal.R×Sc.S.Afr.

Seeds giving rise to hybrid seedlings were smaller and paler than selfed seeds of the mother plant, and this difference was even more pronounced in crosses between *L. saligna* and *L. serriola* than was the case in crosses between *L. saligna* and *L. sativa*.

The hybrids showed much abnormal leaf growth in the early stages, but later they developed into quite vigorous plants especially the hybrids with Sal.C. Somatic chromosome doubling was common in these hybrids, as shown by abnormal leaf growth and presence of side shoots which had buds and flowers bigger than normal, large pollen grains and tetraploid PMC. In the only F_1-hybrid obtained of the combination Sal.C×Sc.L, tetraploid tissue had apparently gained the upper hand at an early stage, as several PMC from different parts of the plant were found to be tetraploid and all flower heads examined had large pollen grains. This plant was presumably wholly tetraploid.

b. *Cytology*

A detailed study of meiosis has been made only in diploid PMC of the hybrid Sal.R×Sc.H. The data in Table 16 show that it was very similar to that of the hybrids between *L. saligna* and *L. sativa*. The number of ring bivalents per cell was even lower than in any of these hybrids.

c. *Fertility*

Data on pollen fertility and seed setting in three combinations are

found in Tables 17 and 18. The fact that somatic chromosome doubling takes place in these hybrids must be expected to have an effect on the fertility. The hybrid Sal.C×Sc.L, which was entirely tetraploid, was found to have good pollen fertility and comparatively good seed setting. A large number of giant pollen grains were seen in pollen samples of the hybrid Sal.C×Sc.H. The hybrid Sal.R×Sc.H appeared to have less tetraploid tissue and had a smaller proportion of giant pollen grains. The results obtained from this hybrid show that pollen fertility and seed setting is poor in hybrids between *L. serriola* and *L. saligna*, unless they have undergone somatic chromosome doubling.

d. F_2 *and later generations*

An F_2-generation of the cross Sal.C×Sc.H, consisting of 19 plants, was raised. No chromosome numbers were determined, but differences in size of vegetative parts and pollen grains made it obvious that there was a variation in chromosome numbers in this F_2. Fourteen of the plants were classified as tetraploid and five as diploid. It is, however, quite possible that some of the presumed tetraploids really were triploids.

The plants classified as tetraploids were more vigorous and had somewhat broader leaves than those classed as diploids, otherwise the family was uniform morphologically with the exception of one very weak plant. The pollen fertility was very variable, that of the tetraploids ranging from 10.3 %—74.0 % with an average of 36.5 %, and that of the diploids from 11.9 %—86.8 % with an average of 39.4 %. Unfortunately no seeds were obtained because the plants were grown out of doors in a very wet summer.

The hybrid Sal.C×Sc.L produced an F_2-generation consisting of 12 plants, all tetraploid. They closely resembled the F_1-hybrids and were very uniform. A line originating from this tetraploid F_2, was found to be practically true-breeding in later generations. A specimen is illustrated in Fig. 67. The fertility has been sufficient to keep the line going without serious difficulties. The following fertility data have been recorded for different generations:

	Pollen fertility		Seed setting	
F_2, field	94.3 %	1 plant	52.3 %	1 plant
F_3, greenhouse	84.2 %	6 plants	28.6 %	4 plants
F_4, „	66.7 %	2 „	37.8 %	1 plant
F_5, „	84.3 %	6 „	11.7 %	3 plants
F_6, „	90.2 %	3 „	13.6 %	2 „

Fig. 67. Tetraploid derivative of the hybrid *L. saligna*×*L. serriola*, Sal.C×Sc.L.

Although the plants with the best seed setting always were used for further breeding, there is no sign of any improvement in fertility with advancing generations.

D. L. sativa×virosa

a. *Description of hybrids*

Crosses between *L. sativa* and *L. virosa* have been successful in both directions, but hybrids were obtained more easily when *L. sativa* was used as the female partner. Hybrid seeds resulting from crosses made in this direction, were smaller and paler than selfed seeds on the same plant (Figs. 46—49). Seeds resulting from crosses with *L. virosa* as mother plant had the purplish black seed coat which is characteristic of *L. virosa*, but the seeds were much smaller and less well developed than those resulting from selfing. Information concerning the viability of the F_1-hybrids has been summarized in Table 19. It has not been possible to produce any viable hybrids between *L. virosa* and any cultivated form of *L. sativa*. In some cases the seeds failed to germinate, in others the hybrids died in the seedling stage. Differences in viability were observed between different combinations. Berl., among the cultivated forms of *L. sativa*, and Vir.K, among the lines of *L. virosa*, gave consistently the most viable hybrids. Accordingly, the hybrids Berl.×Vir.K were more vigorous and survived longer than any of the others (Figs. 68 and 69), but they died before producing a seed stalk.

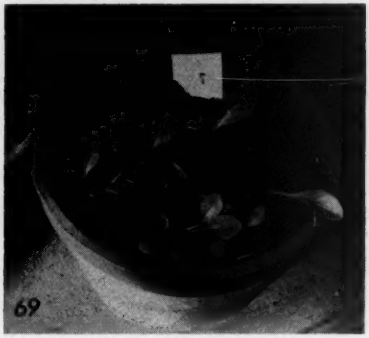
TABLE 19. *Viability of hybrids L. sativa×virosa and L. serriola×virosa.*

Lines of *L. sativa* or *L. serriola*	Lines of *L. virosa*						
	Vir. K	Vir. L	Vir. J	Vir. C	Vir. T	Vir. S	Vir. K × Vir. C
L. sativa							
K. driv	O	O		O			
Asp.	L	L		O			
Berl.	L	L		L			
„ reciprocal		L					
Oakl.	O						
Tezier	L						
R. rouge	L						
Wond.	L						
G. W. Cos			O				
Altaica	N	N		L	L	L	3 N + 4 L
„ reciprocal	O	O		O			2 L
LC	O	L					
SL	N	N				N	
SL reciprocal	O						
SP		D				L	
SP reciprocal		D					
L. serriola							
Sc. H	N	N		N			
Sc. H reciprocal	N	O		1 N + 5 L			
Sc. R	O	N		5 N + 3 L			
Sc. R reciprocal	O			5 N + 1 L			
Sc. S. Afr.	N	N		O			
Sc. J		O					

O = no seedlings obtained N = hybrids with normal vegetative development
L = non-viable seedlings D = Dwarf hybrids

The primitive forms gave very different results when crossed with *L. virosa*. The line LC gave no viable hybrids at all, the line SP gave weak, dwarfed hybrids when crossed with Vir.L in both directions, but Altaica and SL produced in some combinations hybrids with more or less normal vegetative development.

There was a remarkable difference in behaviour of hybrids between Altaica and different lines of *L. virosa*. They all grew vigorously in the early stages, but differences became apparent as the time approached for stem elongation. At this stage the hybrids with Vir.C, Vir.T and Vir.S began to assume an unhealthy crimson colour, growth stopped, the plants began to fade away and died after a few weeks (Fig. 71). No



Figs. 68—69. F_1-hybrids between *L. sativa* and *L. virosa*. — Fig. 68. Berl.×Vir.K. Fig. 69. Berl.×Vir.L.

disease symptoms could be detected. The hybrids with Vir.K and Vir.L continued to grow (Fig. 70) and produced normal seed stalks. They began, however, to wilt away before flowering was finished, hybrids with Vir.L earlier than those with Vir.K. Crosses between Altaica and the F_1 Vir.K×Vir.C produced a total of 3 viable and 6 non-viable hybrids, which suggests that only a few genes are involved in the genetics of hybrid inviability in the crosses between Altaica and *L. virosa*. When used as female partner in crosses with Vir.K, Vir.L and Vir.C, the line SL produced very vigorous and healthy hybrids. No hybrids were obtained when *L. virosa* was used as mother plant for this cross. No

Figs. 70—71. F_1-hybrid between *L. sativa* and *L. virosa*. — Fig. 70. Altaica×Vir.K. Fig. 71. Altaica×Vir.C.

morphological evidence of somatic chromosome doubling was found in any of the hybrids between *L. sativa* and *L. virosa*.

b. *Cytology*

Meiosis has been studied in three hybrids and the results are found in Table 20. Pairing was incomplete in a large proportion of the PMC, and in cells with complete pairing (Fig. 72) the average number of ring bivalents was low which indicates a low chiasma frequency. Bridges were common at I A and later stages. Many of them were without doubt the result of crossing over in inverted segments and were evidence of the existence of structural differences, which were to be expected in view of the morphological dissimilarity of the chromosomes of the two parent species.

No PMC were observed which had the tetraploid chromosome number at I M, but evidence was found of chromosome doubling through non-reduction. The cells pictured in Figs. 73 and 74 probably represent restitution nuclei at II M and II A respectively. A number of dyads were also seen.

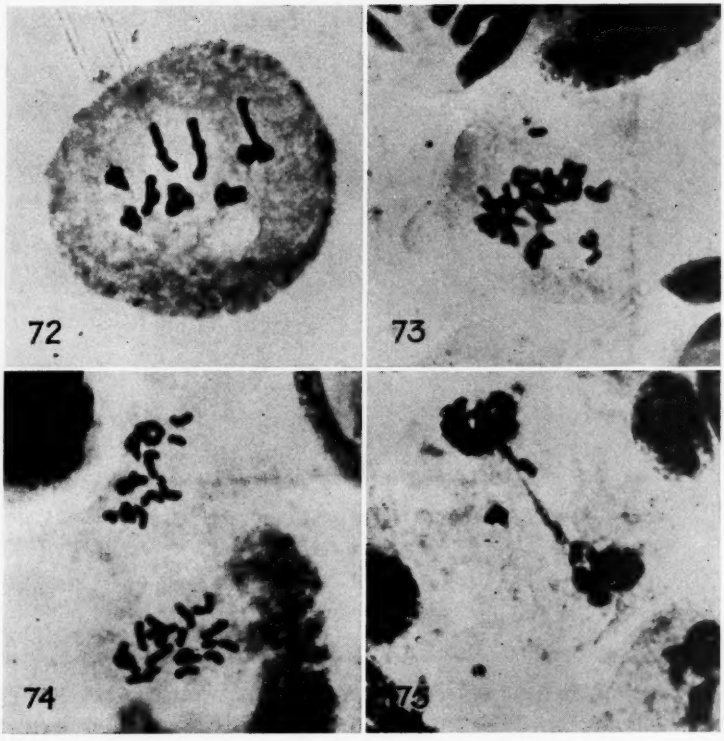
c. *Fertility*

Pollen fertility in hybrids between *L. sativa* and *L. virosa* was very variable and sometimes better than was to be expected in view of the irregularities at meiosis (Table 17). Many of the good pollen grains were very big, presumably diploid. As no evidence of somatic doubling has been found in these hybrids, it must be assumed that these giant pollen grains have arisen through non-reduction at meiosis.

The hybrids were almost completely seed sterile. The percentage of good seeds was determined in two hybrids and in both the figure was below 1 % (Table 18). The seeds were purplish black as in *L. virosa*.

d. F_2 *and later generations*

The few seeds which were produced by the F_1-hybrids failed to germinate or the seedlings died at a very early stage. Tetraploid seeds were, however, obtained from the hybrid SL×Vir.K after colchicine treatment of the young seedlings. A tetraploid F_2-generation was raised from these seeds. It was uniform in morphological characters with the exception of a few plants which had very short seed stalks. A line originating from one of the F_2-plants has shown only very slight morphological variation. A typical specimen is illustrated in Fig. 76. This line has been continued until F_6. The pollen fertility has varied between 49.3 % and 72.5 %, the seed setting between 0.7 % and 2.7 %.



Figs. 72—75. Meiosis in F_1 *L. sativa*×*virosa* and *L. serriola*×*virosa*. — Fig. 72. Altaica×Vir.K; I M with complete pairing. Fig. 73. SL×Vir.L; irregular II M. Fig. 74. SL×Vir.L; irregular II A. Fig. 75. Sc.H×Vir.L; I T with bridge and fragment.

Fig. 76. Tetraploid derivative of the hybrid *L. sativa*×*virosa*, SL×Vir.K.

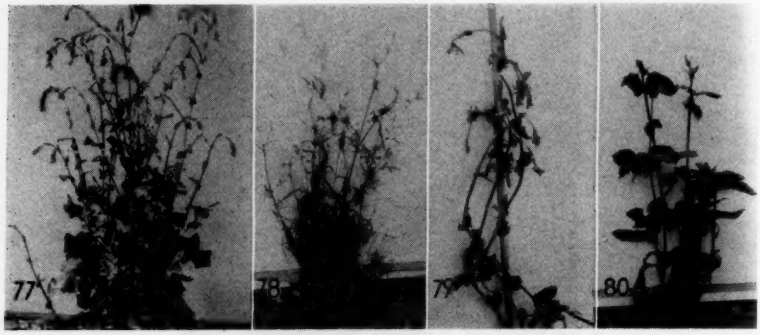
E. L. serriola×virosa

a. *Description of hybrids*

Hybrids between *L. serriola* and *L. virosa* have been obtained in both directions, but more easily with *L. serriola* as the female parent. Hybrid seeds were smaller and paler than selfed seeds, and they germinated badly. Fifteen cross combinations were attempted with the results shown in Table 19. All the F_1-hybrids obtained were of normal vigour (Fig. 77) with a few exceptions which all occurred in crosses where the line Vir.C was one of the parents.

b. *Cytology*

Meiosis was studied in two combinations only (Table 20). Pairing at I M was inferior to that of hybrids between *L. sativa* and *L. virosa*, and the chiasma frequency was lower. As in these hybrids I A and later stages were characterized by lagging chromosomes and inversion bridges (Fig. 77), giving evidence of structural chromosome differences.



Figs. 77—80. F_1 and F_2 of *L. serriola*×*virosa*, Sc.H×Vir.L. — Fig. 77. F_1-plant. Figs. 78—80. F_2-plants.

c. *Fertility*

As in hybrids between *L. sativa* and *L. virosa*, pollen fertility was sometimes surprisingly high (Table 17), and this was associated with the presence of very large pollen grains. As no evidence has been found of somatic doubling, it must be assumed that diploid pollen grains arise through non-reduction also in crosses between *L. serriola* and *L. virosa*. The data in Table 18 show that seed setting was extremely poor. The seeds had the purplish black colour typical for *L. virosa*.

d. F_2 *and later generations*

In spite of the high degree of sterility of the F_1-hybrids, it was possible to raise an F_2-generation of the cross Sc.H×Vir.L. Although the seed setting was not more than 3 %, 369 seeds were collected in a year when weather conditions were favourable for seed ripening. The seeds germinated badly, and only 76 F_2-plants were obtained. Chromosome numbers were determined in eight plants only, and not no root tip squashes, but on parafin sections of flower buds. The quality of these slides was not good enough for exact determination in somatic tissue or in PMC. Two plants were found to have $2n = \pm 36$ and the remaining six $2n = \pm 27$. Thus all these eight plants owed their origin to the functioning of at least one unreduced gamete. There was a tremendous variation in vigour and morphology among the F_2-plants. Many died at an early stage, others developed into semi-dwarfs, but some grew into vigorous specimens. The height of the seed stalks varied between 25 and 155 cm. The two plants with ± 36 chromosomes were very vigorous, those with ± 27 chromosomes were variable. Many kinds of abnormal

TABLE 20. *Meiosis in hybrids L. sativa×virosa and L. serriola×virosa.*

Hybrids	No. of plants	I M									I A+I T			II M			II A+II T		
		Pairing							Ring-rod II										
		No. of PMC with					Number of cells	% PMC with univ.	Average number of ring. biv. per cell	Number of cells	% cells with bridges	% cells with lagg. chrom.	Number of cells	% cells with bridges	% cells with lagg. chrom.	Number of cells	% cells with bridges	% cells with lagg. chrom.	Number of cells
		9 II	8 II 2 I	7 II 4 I	6 II 6 I	≤6 II													
L. sativa × virosa																			
Altaica × Vir. L	2	115	25	6	3	4	153	24.8	5.5	111	22.5	20.0	40	4.9	24.6	122	7.3	16.4	55
„ × Vir. K	1	57	20	4	1		82	30.5	4.8	57	39.3	32.1	28	14.9	27.0	74	25.0	16.7	24
SL × Vir. L	1	67	27	12	6	8	120	44.2	4.7	67	36.0	18.7	75	—	—	—	—	—	—
L. serriola × virosa																			
Sc. H × Vir. L	1	53	32	11	2	2	100	47.0	4.6	53	34.1	15.9	44	—	—	—	—	—	—
Vir. K × Sc. H	1	25	20	4	4		53	52.8	3.6	25	40.0	60.0	5	9.1	59.1	22	33.3	50.0	12

Fig. 81. Tetraploid derivative of the hybrid *L. serriola*×*virosa*, Sc.S.Afr.×Vir.K.

vegetative development occurred (Figs. 78—80). Surprisingly, a normal mendelian segregation was recorded for the characters lobed and non-lobed leaves. Vir.L has the dominant character lobed leaves, and Sc.H the recessive. The F_1-hybrid had lobed leaves and the F_2-generation consisted of 62 plants with lobed and 14 with non-lobed leaves, which is reasonably close to a 3 : 1 ratio ($\chi^2=1.754$). As a contrast, all the 76 plants had anthocyanin colour, although the parent lines differed in this character, Vir.L lacking anthocyanin.

Pollen fertility was very poor in all plants which were examined and giant pollen grains were not rare. Seed setting was extremely poor. Only eight of the plants produced any seeds at all, the highest number per plant being four, and these seeds all failed to germinate. Like the seeds of the F_1-plants, these all had the purplish black colour characteristic of *L. virosa*.

Repeated attempts to backcross F_1-hybrids between *L. serriola* and *L. virosa* with the parents resulted in failure. From a total of 211 pollinated flower heads, only two weak seedlings were obtained, both of which died before they had produced many leaves.

By means of colchicine treatment it was possible to raise an F_2 progeny from the F_1-hybrid Sc.S.Afr.×Vir.K. It consisted of 10 plants of which nine had lobed leaves and conformed to a general type, whereas the 10th was entirely different, having short, non-lobed leaves. Seed setting was very poor, but a line from this tetraploid progeny was carried on until F_4, when it was lost. A specimen is illustrated in Fig. 78. The following details were recorded:

F_3: 11 plants, all conforming to type. Seed setting 1.7 % (average of 3 plants).

F_4: 22 plants, showing segregation in anthocyanin pigmentation and degree of lobing. Pollen fertility 33.2 % (one plant only).

F. L. saligna×virosa

Several attempts have been made to cross these two species, using two lines of *L. saligna* and five lines of *L. virosa*. Pollinations have been made in both directions, but although not less than 397 flower heads in all have been pollinated, not a single hybrid seedling has resulted. It is, of course, possible that the cross may succeed if other biotypes are used.

IV. DISCUSSION

1. Compatibility and viability of F_1-hybrids

The results of this investigation with regard to compatibility and viability of F_1-hybrids may be summarized as follows.

(1) There is no evidence of incompatibility within any of the species belonging to the *serriola* group.

(2) Complete cross compatibility appears to exist between *L. sativa* and *L. serriola*.

(3) Hybrids between *L. saligna* and *L. sativa* or *L. serriola* can be obtained, but only if *L. saligna* is used as the female partner. Matings between *L. saligna* and *L. serriola* or *L. sativa* gave imperfectly developed hybrid seeds. *L. saligna* var. *runcinata* (represented by the line Sal.R) produced non-viable hybrids with all cultivated and some primitive forms of *L. sativa*, but gave viable hybrids with all lines of *L. serriola* and some primitive forms of *L. sativa*. The typical form of *L. saligna*, however, gave viable hybrids with all lines of *L. serriola* and *L. sativa*. Some primitive forms of *L. sativa* gave dwarf hybrids with *L. saligna*. Somatic chromosome doubling is a characteristic feature of hybrids between *L. saligna* and *L. sativa* or *L. serriola*.

(4) Crosses between *L. virosa* and *L. sativa* or *L. serriola* succeed in

both directions. Imperfect development of the hybrid seeds are characteristic also for these crosses. Non viable hybrids resulted from crosses between *L. virosa* and all cultivated forms of *L. sativa*, whereas *L. serriola* and some primitive forms of *L. sativa* gave viable hybrids with *L. virosa*. One of the *L. virosa* lines gave dwarf hybrids with one of the primitive lines of *L. sativa*.

(5) Numerous attempts to cross *L. saligna* and *L. virosa* in both directions all failed.

Hybrids between *L. serriola* and *L. sativa* have previously been produced by many workers, *e.g.* DURST (1930), BREMER and GRANA (1935), ERNST SCHWARZENBACH (1936), THOMPSON, WHITAKER and KOSAR (1941), but hybrids between *L. saligna* and *L. sativa* have not been described previously. This cross was attempted in both directions by THOMPSON, WHITAKER and KOSAR (1941), but without result. These authors obtained hybrids between *L. saligna* and *L. serriola*, but as in the present investigation, the cross was successful only when *L. saligna* was used as the female partner. They also succeeded in crossing *L. sativa* and *L. virosa*, with *L. sativa* as female partner, but their attempts to cross *L. serriola* and *L. virosa* were unsuccessful and also their attempts to cross *L. saligna* and *L. virosa*.

It is interesting that hybrids obtained by THOMPSON, WHITAKER and KOSAR (*l.c.*) between *L. virosa* and the cultivated lettuce variety Imperial D, were viable and extremely vigorous although completely sterile, whereas all hybrids, produced during the present investigation, between *L. virosa* and cultivated forms of *L. sativa*, died as seedlings. ERNST-SCHWARZENBACH (1936) failed to obtain any hybrids in her attempts to cross *L. virosa* with cultivated forms of *L. sativa*.

NAUDIN (1875) has described a spontaneous, fully fertile hybrid between *L. sativa* and *L. virosa*. The facts that artificially produced hybrids between the two species rarely survive beyond the seedling stage and are completely sterile, if they survive, casts some doubt on NAUDIN's identification of the hybrid. His description of the wild parent species fits *L. serriola* equally well as *L. virosa*.

Imperfect seed development, similar to that observed in interspecific crosses in the *serriola* group of *Lactuca*, is known from many other plant genera. A review of this subject has been made by BRINK and COOPER (1947). Seeds, poorly developed, but still capable of germination, may be the result of selfing in normally cross-pollinated species or of crossing races or species with the same or different chromosome numbers. BRINK and COOPER (*l.c.*) emphasized the importance of the

endosperm for seed development and produced convincing evidence for the theory, that abnormal development of the kind referred to here is caused by unsatisfactory growth of the endosperm rather than by weakness of the embryo. The importance of the endosperm in such cases has been pointed out also by other authors, *e.g.* WATKINS (1932), MÜNTZING (1933), HOWARD (1942) and HÅKANSSON (1952). There is every reason to believe that the poor seed development in crosses between species of the *serriola* group is due to partial failure of the endosperm. This is supported by the fact, that similar, imperfectly developed seeds, have been obtained by the present writer also after matings between autotetraploid and diploid *L. sativa*.

The difference in succes of reciprocal crosses between *L. saligna* and *L. sativa* or *L. serriola* may be due to differences in development of endosperm with two genoms from *L. saligna* and endosperm with two genoms from the other species. It has, however, not been proved that fertilization takes place when *L. saligna* is used as the male partner. Therefore the possibility cannot be excluded, that the reason for the failure of the crosses *L. sativa*×*saligna* and *L. serriola*×*saligna* is inability of the pollen tubes of *L. saligna* to develop to the necessary extent in the styles of the other two species. Many cases of this kind of unilateral incompatibility have been reported, *e.g.* by MATHER (1943) in *Petunia*, by MC GUIRE and RICK (1954) in *Lycopersicum*, by HARRISON and DARBY (1955) in *Antirrhinum*. LEWIS and CROWE (1958) showed that this situation normally occurs only in crosses between self-incompatible and self-compatible species, and only exceptionally in crosses between two self-compatible species. As *L. saligna*, *L. sativa* and *L. serriola* are all autogamous, this explanation is therefore unlikely in the present case. As no studies of pollen tube growth have been made, the question must, however, be left open for the present.

A remarkable characteristic of the hybrids between *L. saligna* and *L. sativa* or *L. serriola* is the more or less pronounced tendency to somatic doubling of the chromosome complement. Chromosome doubled offspring of interspecific hybrids in plants, is usually the result of nonreduction at meiosis of the hybrid. Cases of somatic chromosome doubling in interspecific hybrids are, however, known. Classic examples are *Primula kewensis* (DIGBY, 1912) and the amphidiploid resulting from the hybrid *Nicotiana glutinosum*×*tabacum* (CLAUSEN and GOODSPEED, 1925). In these cases chromosome doubling was an exceptional occurrence, but in the *L. saligna* crosses it is a regular feature and the tendency to chromosome doubling persists in F_2 and later generations.

This suggests that it is due to specific gene action rather than to general physiological disturbances in the F_1-hybrid.

Two spontaneous amphi-diploid forms of *Lactuca* have been described by THOMPSON (1942) and THOMPSON, HANEY and KOSAR (1948). Both occurred in hybrids between 17 and 9 chromosome species, namely *L. biennis* ($n=17$) × *L. indica* ($n=9$) and *L. graminaefolia* ($n=17$) × × *L. indica* ($n=9$), but through non-reduction at meiosis of the F_1-hybrids and not through somatic doubling. Evidence of non-reduction at meiosis was in the present investigation found in the hybrids *L. sativa* × *virosa* and *L. serriola* × *virosa*.

Whereas all F_1-hybrids between *L. sativa* and *L. serriola* showed normal development, the viability and vigour of hybrids between these two species and *L. saligna* and *L. virosa* varied according to the choice of parent lines, many combinations resulting in weak or non-viable hybrids.

STEBBINS (1958), in his review of the phenomena of hybrid inviability and weakness, divides the causes into three categories.

(1) Lack of harmony between genes or chromosomes of the parent species. This disharmony may be of a general nature or due to the action of specific genes.

(2) Cytoplasmic or plastid differences between the parent species.

(3) Disharmony between the hybrid embryo and the surrounding tissue.

Category number (1) is recognized by the fact that the crosses are equally unsuccessful in both directions, whereas there is a difference between reciprocal crosses if the cause of inviability is of the kinds included in categories (2) or (3).

The possible reasons for the unilateral success of crosses between *L. saligna* and *L. sativa* has already been discussed. When the cross is performed in the successful direction there are remarkable differences between different combinations of lines of the two parent species. This applies also to crosses between *L. sativa* and *L. virosa*, which can be successfully performed in both directions. The different results obtained with different lines of the same species, must be due to causes belonging to STEBBINS' category no. (1). The available evidence suggests that hybrid inviability in the *serriola* group of *Lactuca* is due to the action of specific genes rather than to general disharmony between the genes of the parent species. The fact that three viable and vigorous F_1-hybrids were obtained out of a total of 9 plants in the cross between Altaica and the F_1-hybrid between the two *L. virosa* lines, Vir.K and

Vir.C, suggests that the number of genes involved is small. This is supported by the very remarkable difference in crossing behaviour between the two *L. saligna* lines, Sal.C and Sal.R, and between different, closely related lines of *L. sativa*, which in other respects are very similar. The situation is reminiscent of HOLLINGSHEAD's classical *Crepis* example where a single gene decides whether strains of *C. tectorum* give viable or non-viable hybrids with *C. capillaris* (HOLLINGSHEAD, 1930). The situation in *Lactuca* is presumably more complicated, at least in the crosses between *L. sativa* and *L. virosa*. Here there are clear differences with regard to the state of development reached by different hybrid combinations before they die. The same was found by SCHMALZ (1959) in his study of subvital hybrids in wheat. He found that the inferior viability of certain hybrids was due to two dominant complementary genes, but was unable to decide whether the differences in vitality between different hybrid combinations was due to the effect of modifying genes or to the existence of different alleles at the two loci.

The results of this investigation suggest, that hybrid inviability in the *serriola* group of *Lactuca* is controlled by complementary genes, some of which are present in *L. sativa* and others in *L. saligna* and *L. virosa*. Certain facts about the distribution of these genes are apparent. Thus the lethal *L. sativa*-genes are present in most of the cultivated forms, and in some, but not all of the primitive forms and they appear to be lacking in *L. serriola*, as the lines of this species have given viable hybrids in all combinations with *L. saligna* and *L. virosa*. Within *L. saligna*, genes for hybrid lethality are present in the line Sal.R, representing the variety *runcinata*, but not in the typical form, represented by the line Sal.C. Similar genes are present in all lines of *L. virosa*. It has not been proved that these genes are identical with those in *L. saligna* var. *runcinata*, but there is a remarkable, although not absolute similarity between Sal.R and the *L. virosa* lines with regard to viability of their hybrids with *L. sativa*. This is shown by the following summary of crossing results, where N means normal hybrids, D dwarfs and L non-viable hybrids.

L. sativa line	Sal. R	*L. virosa* line Vir. K	Vir. L	Vir. C, T and S
Berl.	L	L	L	L
Asp.	L	L	L	—
LC	L	—	L	—
Altaica	L	N	N	L
SP	D	—	D	L
SL	D	N	N	N

Weak, freakish plants, unable to survive beyond the seedling stage, were obtained by THOMPSON, WHITAKER and KOSAR (1941) in a number of crosses between *Lactuca* species, not belonging to the *serriola* group.

Dwarfness in hybrids between *L. saligna* and the primitive *L. sativa* forms SL, SP and SM, appears to be caused by different genes, as dwarf hybrids were obtained with the line Sal.R as well as the line Sal.C. The F_2-results suggest that this dwarf growth is caused by a small number of dominant complementary genes. The three primitive *L. sativa* lines are similar morphologically and the presence of the genes for dwarf growth suggests that they have a common origin, although they have been obtained from three different sources. The line SP produced dwarf hybrids also when crossed with the *L. virosa* line Vir.L, but different genes are presumably responsible in this case, as the SL line gave vigorous hybrids with the same line of *L. virosa*. The dwarfness of hybrids referred to here has many features in common with that found by BERNSTRÖM (1955) when crossing certain lines of *Lamium purpureum* with *L. hybridum*. Similar well known cases are 'crumpled' (HUTCHINSON, 1932) and 'corky' (STEPHENS, 1946) in cotton.

It is unlikely that genes for hybrid inviability or weakness have played any part in the speciation within the *serriola* group, as they are present only in certain strains of the species concerned, and as a high degree of sterility is found in the viable hybrids which are produced between strains lacking such genes. It is more likely that the genes for inviability are the result of changes which have taken place after the species became separated by barriers of other kinds (*cf.* STEBBINS, 1950, 1958, BERNSTRÖM, 1955). This conclusion is supported by the fact that genes of this kind are present in most cultivated varieties of *L. sativa*, but are lacking in the wild species *L. serriola* which is closely related to *L. sativa* and gives fully fertile hybrids with this species. This difference between *L. sativa* and *L. serriola* may, however, have a phylogenetic significance, a question which will be discussed in another connection.

2. Cytology

Distinct differences in chromosome morphology were observed between *L. virosa* and the other three species of the *serriola* group. *L. sativa* and *L. serriola* appeared to have identical karyotypes, but measurements revealed certain dissimilarities between these two species and *L. saligna*.

The somatic chromosomes of *L. serriola*, *L. virosa* and *L. saligna* have

previously been studied by BABCOCK, STEBBINS and JENKINS (1937). They state that the chromosome complements of these three species are very similar to that of *L. sibirica*, *i.e.* they consist of mostly subterminal chromosomes with one large, almost sub-median pair and a small pair of medianly constricted chromosomes. Their illustration of the chromosomes of *L. virosa* is not difficult to reconcile with Fig. 6 in this paper, but the authors do not appear to have observed the difference between this species and the others. This is presumably due to the technique employed. Without the use of oxyquinoline, or some other agent which contracts the chromosomes and inactivates the spindle, it is very difficult to obtain cells where the chromosomes are separated sufficiently to allow accurate study of the chromosome morphology. BABCOCK, STEBBINS and JENKINS point out that they encountered difficulties of this kind.

WHITAKER and JAGGER (1939) found differences between the chromosome complements of *L. serriola* and *L. sativa*, the chromosomes of *L. sativa* being more differentiated. Their observations were made on bivalents at meiosis. It has not been possible to confirm their results and it is unlikely that structural differences of any magnitude should exist, as perfect bivalent pairing has been found in meiosis in hybrids between the two species.

The studies by BABCOCK, STEBBINS and JENKINS (1937), STEBBINS, JENKINS and WALTERS (1953) and STEBBINS (1958 b) have shown that $n=9$ is probably the original basic chromosome number of the *Compositae* tribe *Cichoriae* to which the genus *Lactuca* belongs. In this tribe the possession of panicles with few, large flower heads and achenes with an inefficient dispersal mechanism are regarded as primitive traits. Both allogamous and autogamous species occur, allogamy, which is invariably associated with large flowerheads, being the original breeding system. Primitive species of the tribe have, as a rule, chromosomes which are similar to each other in size and have median or submedian centromeres, whereas advanced species have a more asymmetrical karyotype. There has also been a tendency towards reduction of the chromosome number during the evolution of this tribe, all numbers from $n=9$ to $n=3$ being represented. Within the genus *Lactuca* the above mentioned authors found great variation between different species both with regard to morphological specialization and asymmetry of the karyotype. BABCOCK, STEBBINS and JENKINS (*l.c.*) consider the *Scariola* section as the most advanced sub-genus of *Lactuca* and refer to the three species of the *serriola* group as 'modern, weedy species

with chromosomes very unequal in size and with a majority of subterminal or submedian constrictions'.

According to STEBBINS (1958 b) there is evidence of a causal connection between the evolution of the karyotype in the tribe *Cichoriae* and the breeding system, habitat preference and longevity of the species. In annual species occupying unstable habitats, such as road sides and waste places, and possessing an efficient mechanism for seed dispersal, a genotypical stability is advantageous as such species are able to invade new localities and rapidly build up large populations. Species which quickly can produce a large number of well adapted off-spring are most likely to succeed under such conditions. Genetical stability has in the tribe *Cichoriae* been achieved in some cases by a change of the breeding system to apomixis or autogamy, in others by a reduction of the recombination index which is a function of chromosome number and chiasma frequency. The author emphasizes that both methods are unlikely to be found in the same evolutionary line. In a self-fertilizing species, a high recombination index may, for instance, be an advantage, as the evolutionary potentialities of such a species depends on occasional cross-pollination between genotypically different lines in the populations (*cf.* STEBBINS, 1957) and recombination in the resulting hybrids.

The wild species of the *serriola* group of *Lactuca* are waste place plants, particularly *L. serriola* and *L. saligna*. Genotypical stability is assured by self-fertilization and it is therefore in agreement with STEBBINS's theory that they have retained the original chromosome number of the tribe which, together with a high chiasma frequency, about two chiasmata per bivalent, gives a high recombination index.

STEBBINS (1958 b) also proposed a theory about the evolution of the karyotype within the tribe. According to this the first step is an increase of asymmetry due to pericentric inversions and the second a reduction of the chromosome number through unequal translocation and elimination of small, inert chromosomes.

The species of the *serriola* group of *Lactuca* represent an intermediate stage in this evolution, as they have the same chromosome number as the most primitive species, but karyotypes which have undergone changes resulting in greater asymmetry. These changes may have taken place before self-fertilization became predominant in the group. The fact that there is a higher proportion of chromosomes with subterminal centromeres in *L. virosa* than in *L. serriola* and *L. saligna*, may be due to chance alone. It is, however, interesting that *L. virosa* has much

larger flower heads than the other two species and therefore probably is less completely autogamous. There is, however, no evidence of a reduction of chiasma frequency in *L. virosa*, which might have been expected if a lower recombination index has a selective advantage in the species.

Observations on meiosis in hybrids have confirmed the conclusions drawn from the studies of somatic chromosomes, namely that the chromosomes of *L. sativa* and *L. serriola* are structurally very similar, but that structural differences exist between these two species on the one hand, and *L. saligna* and *L. virosa* on the other. WHITAKER and THOMPSON (1942) also found evidence of structural differences between the chromosomes of *L. sativa* and *L. virosa*.

Meiotic irregularities in the form of univalents at first metaphase and difficulties in separation at anaphase occurred in *L. sativa* more frequently than can be considered normal for an autogamous species. Plants with a high proportion of PMC with univalents had, on the whole, a smaller number of ring bivalents in cells with complete bivalent formation. The regression coefficient, calculated from the data in Table 1, is -0.503 ($P=0.01$—0.001). This indicates that the presence of univalents is due to a general reduction in chiasma formation. The presence of univalents in a certain proportion of the PMC in *L. sativa* has been reported by GATES and REES (1921). Univalents probably occurred also in the material of WHITAKER and JAGGER (1939), although these authors interpreted them as originating from precociously dividing bivalents.

Structures very similar to inversion bridges and sometimes associated with fragments were observed at I A, but it is unlikely that these bridges, even the most persistent of them, were due to crossing over in inverted segments. They occurred not only in hybrids but also in pure lines. Inversions must be expected to occur very rarely in pure lines, and the frequency of bridges in this material was far too high to be accounted for in this way. The anaphase irregularities were on the whole most frequent in plants with a high number of univalents at first metaphase and it is reasonable to assume that the two phenomena have a common cause. There is no reason to doubt the homozygosity of the pure lines, even those in which univalents were most common. The plant of the line SP, for instance, which gave 20.8 % pollen mother cells with univalents, was the result of three generations of propagation by single plant progenies. The original population was perfectly uniform and the line never showed any segregation in morphological

characters. The same applies to the lines SL and LC, which had been propagated in the same manner for five generations.

Environmental conditions have a considerable effect on meiosis. OEHLKERS (1937) and KISCH (1937) found for instance that factors which have the effect of increasing the osmotic pressure, such as drought and heavy applications of fertilizers, seriously reduce chiasma frequency, and GRANT (1952) found that chromosome pairing in *Gilia* hybrids was influenced by the state of nutrition of the plant. There is reason to believe that such factors may have been partly responsible for the abnormalities under consideration, but the meiotic irregularities cannot have been due entirely to environmental factors, as they occurred with variable frequencies in different groups of material grown under similar conditions. Meiosis in pure lines of *L. sativa*, particularly the primitive types, and in hybrids between closely related forms, appear to be more sensitive to changes in environment than meiosis in *L. serriola* and hybrids between less closely related forms. This suggests the presence in *L. sativa* of genes, which make meiosis sensitive to changes in environment.

Many cases are known of genetically determined meiotic abnormalities, and this subject has been reviewed by PRAKKEN (1943). The cases of lack of pairing found in *Lactuca* can be considered as belonging to his category weak asynapsis. In asynaptic plants, lack of pairing at first metaphase is often associated with difficulties in anaphase separation. As examples of this kind may serve the cases described by ANDERSSON (1947) in *Picea*, and by LAMM (1936) and REES (1955) in inbred rye. Cromatin bridges, apparently due to causes other than crossing over in inverted segments, were described by ANDERSSON (*l.c.*) and REES (*l.c.*). REES explains such bridges as due to splitting errors or to genetically controlled chromosome breakage at first prophase of meiosis.

The similarity with the cytological abnormalities found in inbred material and the fact that the irregularities in *L. sativa* occur mainly in pure lines or in hybrids between closely related lines, suggest that they are due to recessive genes. Genes with even a slightly detrimental effect on meiosis must be expected to be largely eliminated in autogamous species by natural selection (MÜNTZING and AKDIK, 1948; LAMM, 1936), but it is perhaps not surprising that they should have survived to a certain extent in a cultivated species where at least a slight reduction in reproductive capacity is without importance (*cf.* SCHWANITZ, 1957 p. 22). Seed setting in pure lines of *L. sativa* is rarely perfect, even under favourable conditions, but if the reason for this were failure of gametes

with a chromosome complement, unbalanced due to irregularities at meiosis, a corresponding reduction in pollen fertility would be expected. A comparison between the data in Tables 2 and 3 shows that such is not the case, the pollen fertility being considerably better than the female fertility. The data in Tables 1, 6 and 12 do not indicate that there is any correlation between seed setting and proportion of PMC with univalents ($r = -0.002$). Moreover, the seed producing potentiality of a lettuce plant is such, that a very large number of seeds is produced in spite of the observed proportions of undeveloped ovules. It is therefore obvious that the meiotic disturbances can only have a very slight effect on reproductive capacity. It is, however, interesting to note that the three wild species of the *serriola* group appear to have a more stable meiosis. The comparatively frequent occurrence of irregularities in the primitive *sativa* forms suggests that these are not truly wild types.

Although it has been shown above that the evidence favours the conclusion that the bridges observed at I A in hybrids within *L. sativa* are not as a rule due to structural differences, the possibility cannot be entirely excluded that some of these bridges are, in fact, due to crossing over in inverted segments.

No multivalents were found in any of the interspecific hybrids in the *serriola* group. This indicates that the differences in chromosome morphology between the different species are due to pericentric inversions and not to translocations between different chromosomes. Translocations appear to be very rare in this group. A case was reported by COOPER and MAHONY (1935) in *L. scariola* var. *integrata* (=*L. serriola* f. *integrifolia*), but WHITAKER and JAGGER (1939) and the present writer have been unable to confirm this finding and it is presumably an exceptional occurrence.

GATES and REES (1921) found frequent associations of more than two chromosomes at metaphase of meiosis in *L. sativa*. These associations are presumably not due to translocations, as they were frequent at metaphase, but occurred only occasionally at diakinesis. The authors explain them as due to end to end coalescence of bivalents.

Differences between lines with regard to satellite size were found within *L. serriola*, *L. sativa* and *L. saligna*, but they could not be correlated with any morphological or physiological differences. LESLEY and LESLEY (1935), LESLEY (1938) and GOTTSCHALK (1953) have studied variation of satellite size in commercial varieties of tomato. LESLEY (1938) found that increase in satellite size was caused by addition of

satellite material from other chromosomes. BOSE (1957), in inbred rye, found variation in satellite size as well as in length of the chromosome arms. He considered this to be caused by addition or deletion of heterochromatin. As the variation of satellite size in *Lactuca* does not appear to be accompanied by any genetical differences, it may well be due to differences in the amount of heterochromatin present, but no cytological evidence is available on this point.

Variation in satellite size has been found also in the wild species *L. serriola* and *L. saligna*, but the data available at present do not suggest that the character has any phylogenetic significance. They indicate, however, that satellite size is normally constant for plants of the same population of the wild species and that different populations differ in this respect. Further studies may reveal trends of importance for the understanding of how the species have spread from one region to another. It has been shown that satellite size can be used as a chromosome marker in segregating families, and it may thus in due course be possible to identify linkage groups with the satellite chromosomes.

3. Fertility

It is reasonable to assume that at least some of the cases of reduced pollen fertility found in pure lines of *L. sativa* are the result of the irregularities observed at meiosis. A certain connection between the two phenomena can be discerned. For instance, the two lines Tezier cos and SP, in which the lack of pairing was most pronounced, were also those in which plants with reduced pollen fertility occurred most frequently. There is, however, no significant correlation between pollen fertility and the proportion of PMC with univalents in those particular plants in which meiosis was studied ($r=-0.2526$; $P>0.2$), but a strong correlation is hardly to be expected if the degree of irregularity at meiosis is influenced by environmental conditions, as no attempt was made, when taking the pollen samples, to choose flower heads which had passed through the meiosis stage at the same time as the buds which had been used for the cytological studies.

The partial pollen sterility observed in hybrids within *L. sativa* and between *L. sativa* and *L. serriola*, must be of a different nature, as meiosis is more regular in the hybrids, particularly in the *L. sativa*$\times$ $\times$*serriola* hybrids, and as the partial sterility is correlated with disturbed segregation of certain genes. The nature of this sterility will be discussed in another connection. The possible effect of the meiotic

irregularities on seed setting in *L. sativa* has already been discussed. Seed setting in this species has previously been studied by JONES (1927) who found 88.5 % seed fertility in the variety Iceberg grown out of doors in California, and by EINSET (1944) who states that in his experiments several diploid varieties were fully fertile under greenhouse conditions.

In the present investigation seed setting in F_1-hybrids between different lines of *L. sativa* and between *L. sativa* and *L. serriola* was found to be markedly superior to that of the parents, particularly under unfavourable conditions. The reason for this is not clear, but three possible explanations suggest themselves:

(1) Better functioning of the selfpollination mechanism in the F_1-hybrids.
(2) Better growth of the pollen tubes in the F_1-styles.
(3) Better development of the seeds on the F_1-plants.

BERNSTRÖM (1950) found improved fertility in intraspecific hybrids in *Lamium purpureum* and *Lamium hybridum* and was in favour of the explanation that the selfpollination mechanism functions better in the F_1-hybrids than in the parents.

EINSET (1944) studied seed setting in diploid and autotetraploid *L. sativa* and found complete fertility in the diploids but very low fertility in the tetraploids. The reason for the sterility of the tetraploids was partly breakdown of the meiotic process in about 20 % of the ovules, and partly failure of pollen grains to germinate and grow in the styles. A tetraploid F_2-hybrid was compared with its tetraploid parents and was found to have a much better fertility. EINSET explains this as due to the beneficial effect of heterozygosity on the general physiology of the plant. The data presented here show, that also in diploid forms there is a correlation between heterozygosity and seed setting, at least under adverse conditions. There is thus a certain similarity with EINSET's results from autotetraploid lettuce, but whether the same factors are operative is an open question.

The possibility must also be considered that the improved seed setting of the F_1-hybrids is due, not to a higher proportion of fertilized ovules, but to better seed development after fertilization. In the discussion above about the defective seeds obtained after interspecific matings, attention was drawn to the importance of the endosperm for seed development. In this connection the results quoted by BRINK (1952) from *Medicago sativa* are of the greatest interest. In this species seed develop-

ment was found to be much better after cross-pollination than after selfing and it was conclusively proved that this was due to better growth of the endosperm. BRINK's explanation is, that after selfing the endosperm, rather than the embryo, suffers from an inbreeding effect. If this explanation is applied to the present case, it would mean that the endosperm in seeds of the pure lines of *L. sativa* carries recessive genes with a slight detrimental effect. Although the endosperm in the seeds on the F_1-plants represents the F_2-generation, it must be expected to be fairly heterozygous in most cases, particularly as it is the result of fusion of three nuclei. This explanation is supported by the fact that other evidence has been found in *L. sativa* for presence of recessive genes with an effect simulating a slight inbreeding depression, namely meiotic irregularities in pure lines and an indication of a heterotic effect on some characters in F_1-hybrids.

In hybrids between *L. sativa* or *L. serriola* and the other two species of the group, *L. saligna* and *L. virosa*, fertility was very low, except in cases where chromosome doubling had taken place. The seed setting was particularly poor and this can hardly be entirely accounted for by the irregularities at meiosis and their effect on gametic viability as judged by the percentage of apparently good pollen. It is possible that poor development of the endosperm may have played a part also in this connection.

4. Relationship between the species

The present investigation has amply confirmed the finding by several previous authors, that no barriers to gene exchange exist between *L. sativa* and *L. serriola*. Barriers of sterility and hybrid inviability and weakness exist, however, between these two species and the other species of the group. Fertile derivatives have been recovered from the cross *L. saligna*×*L. sativa* in spite of the high degree of sterility of the F_1-hybrid. No such fertile derivatives have been obtained from crosses between *L. sativa* or *L. serriola* and *L. virosa*. In the F_2-generation raised from a cross between *L. serriola* and *L. virosa*, all the plants were found to be almost completely sterile. The few plants for which chromosome numbers were determined, had all arisen through the functioning of at least one unreduced gamete. According to personal communication by Dr. E. J. RYDER of the United States Department of Agriculture, American breeders have succeeded in transferring genes from *L. virosa* to *L. sativa*, but this was possible only by devious means, involving the

use of colchicine for chromosome doubling (*cf.* also leaflet by U. S. Dept. of Agr., 1958).

On the basis of these results and the definitions given by CLAUSEN, KECK and HIESEY (1945) and CLAUSEN (1951 p. 171) the four taxonomic species of the *serriola* group must be considered as belonging to the same cenospecies, which comprises the three ecospecies *L. sativa—serriola, L. saligna* and *L. virosa. L. sativa* and *L. serriola* must be considered as ecotypes of the same ecospecies. *L. virosa* is, however, so distantly related to the other two ecospecies that one might be justified in considering it as a separate cenospecies. As was pointed out in the introduction, the *serriola* group occupies an isolated position within the genus *Lactuca.* CLAUSEN, KECK and HIESEY (1945 p. 102) gave this group the rank of comparium as it has not been possible to obtain any hybrids between any of its members and other species of *Lactuca.* This is, however, not strictly correct as THOMPSON, WHITAKER and KOSAR (1941) obtained a partly fertile hybrid between *L. virosa* and the 17 chromosome species *L. graminifolia* MICHX.

The following relationship order is suggested for the species of the *serriola* group:

Ecospecies	Ecotypes
L. saligna	
L. sativa-serriola	*L. sativa,* cult. var. „ „ , primitive types *L. serriola*
L. virosa	

The crossing results suggest for the *L. sativa-serriola* ecospecies a position between *L. saligna* and *L. virosa.* This is supported by the fact that the wild species of this group, *L. serriola,* is morphologically intermediate between *L. saligna* and *L. virosa.* The order between the three ecotype groups of the ecospecies *L. sativa-serriola* is based on the ease with which hybrids are obtained with *L. saligna* and *L. virosa* respectively, and on the viability and vigour of the hybrids. It would be interesting to know the biosystematic position of the Middle East species *L. aculeata* BOISS. and KY. and *L. scarioloides* BOISS. which have many morphological features in common with *L. serriola.*

Fertile hybrid derivatives, originating from highly sterile F_1-hybrids like those recovered by the writer from the cross between *L. saligna* and *L. sativa* have been reported by a number of authors. STEBBINS (1950, p. 286) mentions a number of such cases and points out that they may give rise to new species, if they form sterile or partly sterile hybrids

with either parent. EPLING (1947) gives evidence which suggests that the species *Delphinium gypsophilum* may have originated in this way.

The fertile derivatives of the hybrid *L. saligna*×*sativa* may be the means of transferring genes from one species to another, which would make it possible to compare the identity of genes in the two species. Hybrids between *L. saligna* and *L. sativa* may also be of practical importance, particularly as *L. saligna* appears to be much more resistent than *L. sativa* or *L. serriola* to attacks by fungi such as *Botrytis cinérea* and *Bremia lactucae*.

In spite of the fact that somatic chromosome doubling is very common in hybrids between the *L. sativa-serriola* ecospecies and *L. saligna*, no amphidiploids exist in nature. Evidence that spontaneous hybrids sometimes occur, has been found by the writer in a herbarium specimen from Palermo, Italy, which is illustrated in Fig. 57. This specimen showed the inflorescence typical of F_1-hybrids between *L. saligna* and *L. sativa* or *serriola*. Amphidiploids must therefore be expected to arise occasionally, but they are apparently not successful in nature. This is in agreement with the fact pointed out by CLAUSEN, KECK and HIESEY (1945, p. 118) that most successful amphidiploids have arisen through gametic doubling, not through somatic doubling.

SUMMARY

(1) The four taxonomic species of the *serriola* group of *Lactuca* form three ecospecies, namely *L. sativa-serriola*, *L. saligna* and *L. virosa*.

(2) Only small differences in chromosome morphology exist between *L. sativa-serriola* and *L. saligna*, but the chromosomes of *L. virosa* are markedly different. Variation in satellite size has been observed between lines within *L. sativa-serriola* and *L. saligna*.

(3) *L. sativa-serriola* can be crossed with *L. saligna* only when the latter species is used as female partner, but with *L. virosa* in both directions. It has not been possible to cross *L. saligna* and *L. virosa*. Matings between different ecospecies result in defective seeds.

(4) Line differences exist within all three ecospecies with regard to complementary genes, producing inviability or dwarfness in interspecific hybrids.

(5) Somatic chromosome doubling is a feature of hybrids between *L. sativa-serriola* and *L. saligna*. Non-reduction at meiosis has been observed in hybrids between *L. sativa-serriola* and *L. virosa*, but not somatic doubling.

(6) *L. sativa-serriola* has given fertile, diploid, hybrid derivatives with *L. saligna*, but not with *L. virosa*.

(7) Recessive genes with a slightly deleterious effect on meiosis and on seed setting, simulate a slight inbreeding depression in *L. sativa*.

(8) Partial pollen sterility, not connected with any meiotic irregularities, has been encountered in hybrids between different lines of the *L. sativa-serriola* ecospecies. This partial sterility is correlated with abnormal segregation of certain genes.

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RADIATION RESPONSE OF SOAKED BARLEY SEEDS

I. SUBSTANCES LOST BY LEACHING [1]

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I. INTRODUCTION

SOAKED seeds have been shown to be damaged more by X-rays than non-soaked seeds (PETRY, 1921; STADLER, 1928; GUSTAFSSON, 1937, 1947; GELIN, 1941; EHRENBERG *et al.*, 1953). From these studies it was widely accepted that the radiation susceptibility of a biological system increases with increasing water content. Recently, however, it was shown that the relationship between radio-sensitivity and water content may be influenced by secondary factors. For example, with very dry seeds an inverse relationship between the water content of seeds and their X-ray response was demonstrated (CALDECOTT, 1955; CURTIS *et al.*, 1958). This inverse relationship was related to the after-effects of X-radiation.

In the many investigations (cf. above) on the relation of germination and soaking to seed radiosensitivity the influence of changes in water content has received the prime consideration. Until now the role of substances lost in the leachate (water used for soaking or germinating seed) has not been considered as a factor in seed radiosensitivity. This study was undertaken firstly, to identify the organic and inorganic substances lost from variously soaked irradiated and non-irradiated seeds (results

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to be reported herein) and secondly, to determine if the kinds and amounts of substances lost are related to the biological damage in seeds following various soaking and irradiation treatments (results to be reported in a subsequent paper). The results should be of considerable value since most of the current radiobiological studies using seeds, *e. g.* barley, require seed soaking and/or germination after irradiation.

A few workers in the malting industry have studied some of the substances lost in the liquor from steeped barley seeds. COOK and POLLOCK (1952) found amino butyric acid, alanine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, pipecolinic acid, phenylalanine, proline, threonine, tyrosine, serine and valine in the first steeping liquor. They (1953) isolated various fractions of steep liquor and showed that at least 0.03 per cent of the weight of the seed was removed, largely as free amino acids. Tests for soluble carbohydrates of barley kernels showed sucrose, raffinose, glucose, fructose, maltose and glucodifructose (KLUYVER, 1914; MACLEOD, 1951, 1952, 1953, 1957; GILLES *et al.*, 1952; PREECE and MACLEOD, 1953; SCHMIDHAUSER, 1955) to be present. Non-germinated seed contained, in addition to simple sugars, oligosaccharides and high molecular fructosans, a series of compounds of the order of molecular magnitude of 10 hexose units. A water-soluble crude polysaccharide (barley gum) containing xylose, glucose and arabinose was also noted (GILLES *et al.*, 1952). COOK and POLLOCK (1953) demonstrated the presence of three free sugars (probably sucrose, arabinose and rhamnose) and gummy substance in the first steeping liquor.

Inorganic constituents comprise about 3 per cent of the total weight of barley seeds. The elements shown to be present in barley seeds are: 54 to 119 ppm. of Zn (BERTRAND and BENZON, 1928; BERGH, 1943; BOSTICCO, 1953 a), 6.2 to 11.9 ppm. of copper (GREAVES and ANDERSON, 1936; BOSTICCO, 1953 b), 12 to 20 ppm. of manganese (GREAVES and HIRST, 1929; HYDE, 1948), 1,000 ppm. of sulphur (SCHILD and JACOB, 1953), a trace of boron (WARINGTON, 1923) and phosphorus (VANLAER and DUVINAGE, 1923). LOPEZ DE AZCONA *et al.* (1949) spectrographically demonstrated the presence of aluminum, barium, calcium, copper, iron, potassium, lithium, magnesium, manganese, molybdenum, sodium, nickel, phosphorus, silicon and titanium. The mineral composition of the steeping liquors is not well known. VANLAER and DUVINAGE (1923) reported a 10 per cent loss of phosphorus during steeping.

MASSART (1957) has recently shown that many substances are lost from isolated barley embryos to the water culture. Lost substances

included sucrose, fructose, glucose, and a trisaccharide (possibly a glucodifructose), alanine, glutamic acid, tyrosine, valine, citrulline, leucine, threonine, ornithine, lysine, serine, proline and peptides. One-sixth of the embryonic nitrogen was lost in the form of amino acids. The application of coumarin to inhibit sprouting did not interfere with the leaching process (MASSART, 1955).

The aleurone and embryo contain 75 per cent of the sucrose, 80 per cent of the raffinose, 65 per cent of the glucodifructose (PREECE and MACLEOD, 1953) and 50 per cent of the protein in the barley grain (POSTEL, 1957); the remainder of the sugar and protein fractions were present in the endosperm. The embryo contains two-thirds and endosperm one-third of the total amount of free sugars in the barley seeds. However, raffinose and sucrose form the main part of the embryo, whereas maltose and fructose were the major sugars of the endosperm (SCHMIDHAUSER, 1955).

The differentially permeable nature of the envelope covering the barley grain (pericarp & testa) has been studied in many steeping experiments (BROWN, 1908, 1909, 1917; GRÜSS, 1929, 1930). COLLINS (1918) and THARP (1935) showed that the barley grain, with the exception of micropyle and suberized resistant tissue of the chalazal tract, is completely covered with a strong cutinized envelope. Moreover, the cutinized layer is rather thin over the embryonic region. Evidently only a very slight amount of water absorbed during steeping enters by the general surface of the grain, and most of the water was found to enter at the germinal region (THARP, 1935). BROWN (1943) found that the rate of water absorption and gaseous exchange of the seed embryo was less than that of the isolated embryo and attributed this to the semi-permeable nature of the seed covering.

Recently, several investigations have demonstrated chemical changes in various organisms following X-radiation. Each of these investigations used very high doses of X-ray (100 to 600 Kr). EHRENBERG and JAARMA (1955) observed an increase in the total reducing sugars of resting barley seeds following X-radiation. This change was attributed to the decomposition of the endosperm starch. Further experimentation (1957) showed that the number of breaks produced in polysaccharide chains of the starch molecules per energy absorbed decreased with increasing water content of barley seeds.

Chemical changes from high doses of X-radiation have also been detected in ciliates (CLARK, 1958) and yeast (O'BRIEN, 1958). Many amino acids leached into the surrounding medium when ciliates were

x-rayed at 100 and 600 Kr. No such leakage was observed in non-irradiated animals (CLARK, 1958). In x-rayed yeast, on the other hand, a greater loss of phosphorus in the suspending medium was detected (O'BRIEN, 1958). Potassium and UV absorbing compounds were also released into the medium.

II. EXPERIMENTS WITH 1956 SEEDS

Barley (*Hordeum vulgare*, hulless variety Himalaya CI 620) seeds (caryopses) of 1956 crop were used for first series of experiments reported below. Spikes were threshed by hand and only unbroken fully formed seeds with approximately 8 per cent moisture (obtained by storage in desiccators over dry $CaCl_2$) were used.

Separate lots of 1,050 seeds were soaked in distilled water for different time intervals (0, 2, 4, 6, 8, 10, 12, 20 and 24 hours) at 20° and 30° C. while either nitrogen or oxygen was continuously bubbled through the water. Hereafter to simplify terminology, seeds soaked in the above manner will be called oxygen-soaked or nitrogen-soaked. Control seeds were either not soaked or soaked in oxygen-free distilled water (pH approximately 5.8), boiled for at least 15 minutes and then cooled with nitrogen gas bubbling through it. Seeds were quickly dried with fan forced air for about half an hour and then placed over a saturated solution of KNO_3 and NH_4Cl to bring the moisture content of seeds to equilibrium at approximately 15 per cent moisture.

A. Chemical analysis of leachates

Oxygen-free distilled water (75 ml. for 1,050 seeds) in which presoaking treatments for various periods of time were given was analysed for organic substances. At the end of the soaking period the total volume of the leachate was adjusted to 75 ml. by adding distilled water since some water was always lost owing to absorption by the dry seeds.

Chemical constituents for which the leachates were tested were:

1. *Total ninhydrin positive substances*

These were determined by using an aliquot (0.1 ml.) of the original leachate from each treatment. Photometric determinations were made at 570 mμ using a Beckman DU Spectrophotometer (TROLL and CAN-

TABLE 1. *Total ninhydrin positive substances in barley seed leachate obtained for several periods of time in presence of nitrogen or oxygen at 20° C.*

(Photometric determination using Beckman DU Spectrophotometer)

Leaching treatments	Optical density at 570 mμ	Leucine equivalent μm/ml.	Leucine equivalent μm/seed
.25 hr. nitrogen	.268	1.20	.086
.50 hr. „	.364	1.65	.118
1.00 hr. „	.339	1.58	.113
1.50 hr. „	.415	1.85	.132
2.00 hr. „	.444	2.00	.143
4.00 hr. „	.421	1.90	.136
6.00 hr. „	.953	4.35	.311
12.00 hr. „	.787	3.54	.253
24.00 hr. „	.697	3.24	.231
3.00 hr. oxygen	.868	3.95	.282
6.00 hr. „	.923	4.20	.300
9.00 hr. „	.921	4.20	.300
12.00 hr. „	.603	2.75	.196
24.00 hr. „	.238	1.07	.076

Leucine Standard Solution of 1μm/ml was used.

NAN, 1953). The results obtained were expressed as μ moles leucine equivalents (Table 1).

Ninhydrin complexes of substances containing free amino acids, peptides, protein and ammonia were lost from the seeds during soaking. This loss was initiated in less than 15 minutes (Table 1). The amount increased with soaking time and the maximum amounts were recovered at six hours of soaking in either oxygen or nitrogen at 20° C. After six hours and up to twenty-four hours, the concentration of these compounds decreased in both leachates.

2. *Amino acids*

Twenty ml. of the original leachate were concentrated ten times by lyophilization. For qualitative determinations, fifty λ (micro liters) of the concentrate were spotted on Whatman No. 1 paper and two dimensional paper chromatograms were made using phenol: water (80 : 20, w/w) and n-butanol : acetic acid : water (50 : 15 : 35, v/v/v) as first

TABLE 2. *Estimation of amino acids by quantitative paper chromatography. Micromoles leucine equivalent of amino acids from 50 λ concentrate (equivalent to 0.5 ml leachates) of barley seed leachates obtained for several periods of time in presence of oxygen or nitrogen at 20° C.*

Amino acids	Wave Length mμ	Optical density							
		Nitrogen						Oxygen	
		.25 hr	.5 hr	1 hr	2 hr	6 hr	24 hr	6 hr	24 hr
α-alanine	570	.08	.098	.090	.12	.315	.05	.258	.105
γ-amino butyric acid	570	.015	.015	.02	.018	.188	.010	.195	.057
arginine	570	.105	.135	.14	.12	.11	.077	.028	absent
asparagine	570	.01	.01	.01	.01	.077	.033	.04	absent
aspartic acid	570	.01	.016	.037	.016	.068	absent	.14	.033
glutamic acid	570	.031	.037	.05	.044	.60	.065	.37	.052
glycine	570	.008	.01	.01	.01	.04	.029	.028	.02
leucine-isoleucine	570	.044	.046	.02	.04	.09	.099	.145	.02
methionine sulphoxide	570	.013	.015	.01	.01	.007	.04	.06	.01
proline	440	.015	.02	.01	.019	.078	.048	.035	.023
serine	570	.044	.048	.057	.04	.180	.122	.106	.015
threonine	570	.008	.013	.019	.015	.07	.057	.052	.008
valine	570	.048	.05	.057	.048	.015	.146	.157	.015

and second solvents, respectively. The chromatograms were dried with hot air and sprayed with 0.1 per cent ninhydrin in 95 per cent alcohol. Amino acids were identified according to a technique developed by Dr. C. G. WOODBRIDGE, Horticulture Department, Washington State University, Pullman.

For semi-quantitative determinations of amino acids, ninhydrin spots on chromatograms corresponding to individual amino acids were cut out and the color eluted with 10 ml. of 75 per cent alcohol. Optical density was determined at 570 mμ using a Colman Junior Spectrophotometer. Standard graphs were obtained with known amounts of leucine and the results were expressed as μm leucine equivalents (Table 2).

Within 15 minutes leachates contained all the amino acids in small quantities (Fig. 1 a). These amino acids reached a maximum in 6 hours leachate (Fig. 1 b) and included: α-alanine, γ-amino butyric acid, arginine, asparagine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, methionine sulphoxide, proline, serine, threonine, valine and 2 to 3 unknowns.

The amino acid concentration expressed in terms of leucine equivalents in the leachates increased with time of soaking and reached a maximum at 6 hours in both oxygen and nitrogen (Table 2). It decreased sharply afterwards and very little of the amino acids were present at 24 hours, especially in the presence of oxygen.

3. *Sugars*

Qualitative estimations for sugars were made from one dimensional chromatograms using n-butanol : pyridine : water (6 : 4 : 2, v/v/v) as the solvent. Chromatograms were brushed with diphenylamine: aniline: phosphoric acid reagent (BUCHAN and SAVAGE, 1952). Desalting of samples with Dowex 50 Cation exchange and Dowex I Anion exchange resins before chromatography gave better results for unknowns. Very small amounts of glucose and fructose were present in both oxygen and nitrogen leachates (Fig. 2).

4. *Organic acids*

Qualitative estimations for organic acids were made from one dimensional chromatograms using solvent rich phase of n-butanol : formic acid : water (90 : 63 : 90, v/v/v). The chromatograms were sprayed with bromo cresol green (LUGG and OVERELL, 1948).

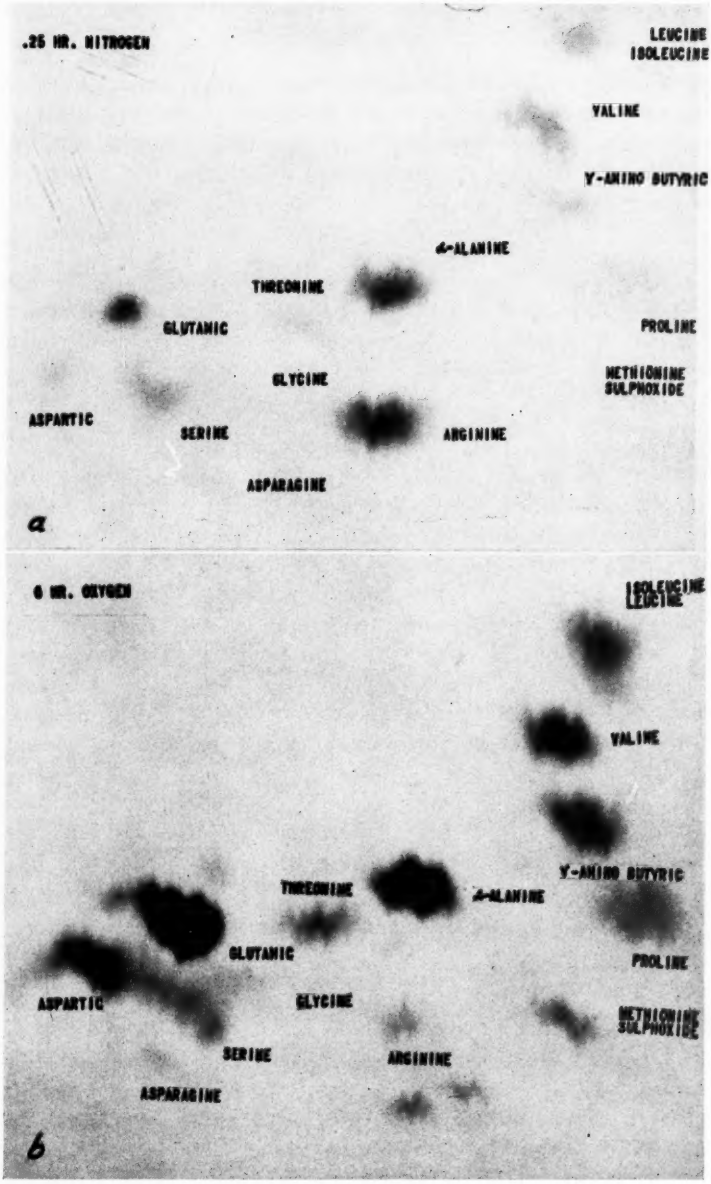

Fig. 1. — a: Two-dimensional chromatogram showing the presence of many amino acids in a leachate obtained by soaking barley seeds for 0.25 hours in the presence of nitrogen at 20° C. — b: Two-dimensional chromatogram for amino acids in a leachate obtained by soaking barley seeds for 6 hours in the presence of oxygen at 20° C.

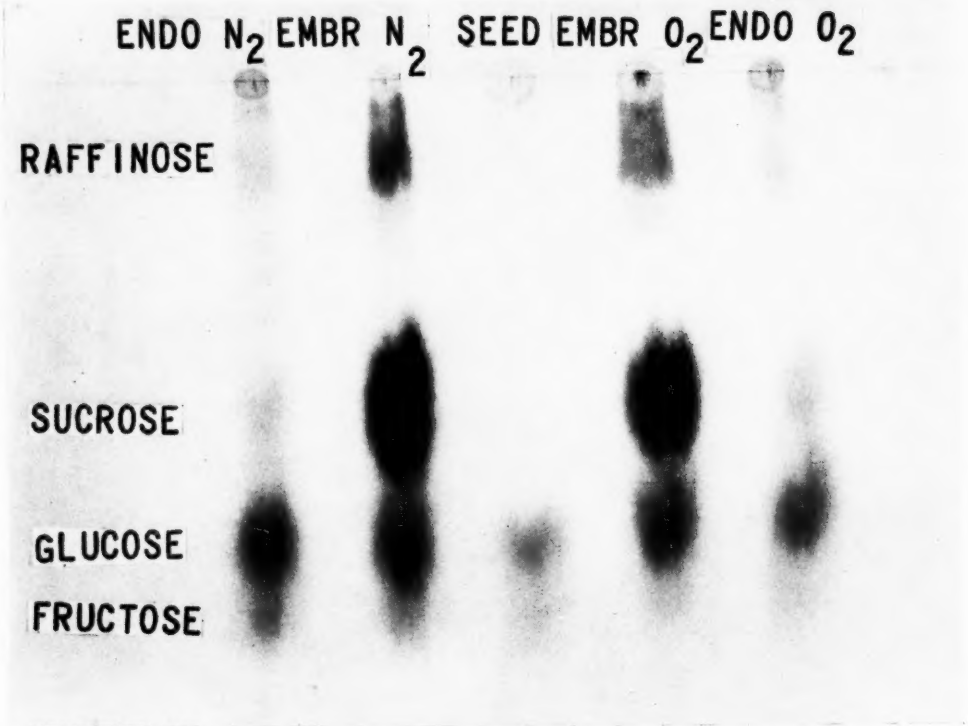

Fig. 2. One-dimensional chromatogram for sugars in 6-hour leachate from barley whole seeds, isolated embryos and endosperms. The samples from left to right are leachates from endosperms, embryos and whole seeds in the presence of nitrogen, and leachates from embryos and endosperms in the presence of oxygen at 20° C.

Two unidentified organic acids were found in oxygen and nitrogen leachates. Comparison with several known organic acids on chromatograms indicated that the unknowns did not match with citric, succinic, α-ketoglutaric, malic, maleic or oxalic acids.

5. *Fatty acids*

Ether extracts of the leachates were used in paper chromatography for fatty acids. The method followed was that of GABY *et al.* (1957)

No fatty acids were detected. However, a small amount of oily substance separated from leachates on freezing as a water immiscible phase.

6. *Sugar phosphates*

One dimensional chromatograms for sugar-phosphates were made on Whatman paper No. 1 that had been washed twice with 2N acetic acid and then distilled water. The solvent used in chromatography was n-propanol : NH_4OH : water (60 : 30 : 10, v/v/v) and spraying technique was the same as HANES and ISHERWOOD (1949).

All of the leachates contained inorganic phosphate and possibly one or more unidentified sugar phosphates. The mobility of the unknown substance on paper did not correspond to glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, fructose 1, 6-diphosphate and ribose-5-phosphate which were chromatographed along with leachates.

7. *Other UV absorbing compounds*

A mineral light lamp (Model SL) was used to scan the unsprayed chromatograms, developed for sugar phosphate study, to reveal any purine or pyrimidine containing compounds. However, none were found in the leachates.

B. Chemical analysis of hydrolysed leachates

Leachate was hydrolysed by heating in a boiling water bath with 6N HCl for 1 hour. This hydrolysate was studied by paper chromatography for sugars, sugar phosphates and organic acid as described above. The hydrolysed leachate showed two additional sugar phosphates and three additional organic acids. One of these organic acids was oxalic acid. Thus it appears that some of the substances are present in the leachate in a bound form.

III. LEACHING EXPERIMENTS WITH 1958 SEEDS

For the following series of experiments, seeds of the 1958 crop were used.

A. Inorganic constitution of the leachates

Lots of 1000 seeds were soaked for 6, 12, 20 and 24 hours in nitrogen at 20° C. Leachate was dried over a steam bath in a platinum dish and ashed at 600° C. for 2 hours. The analyses for inorganic elements were conducted using 5 mg. of the ash by means of the mass spectograph

TABLE 3. *Spectographic analysis for inorganic constituents (ppm of ash) in barley seed leachates* [1] *obtained from 12, 20 and 24 hour soakings in nitrogen at 20° C.*

Sample	Leachates			Whole seed (ppm of ash)
	12 hr.	20 hr.	24 hr.	
Al	1150	1250	850	400
Ba	30	30	20	100
Ca	5000	5000	5000	3000
Cr	5	5	5	20
Co	x	x	x	x
Cu	150	150	115	300
Fe	600	600	500	2000
Pb	x	x	x	x
Mg	190	190	190	5000
Mn	800	800	700	2000
Mo	x	x	x	80
Ni	50	50	50	20
P	1000	1000	1000	1000
K	5000	5000	5000	10000
Si	5000	7000	2000	3500
Ag	x	x	x	16
Na	5000	5000	5000	5000
Sn	x	x	x	x
Sr	80	50	50	100
Ti	40	100	20	T
V	x	x	x	x
Zn	100	100	100	100

[1] Double distilled (Pyrex) water was used for soaking seeds.
The following abbreviations have been used: x=Absent; T=Traces detectable.

in the Industrial Engineering Laboratories of the Washington State University, Pullman.

Spectrographic analyses revealed many inorganic elements in the leachates. These included: aluminum, barium, calcium, chromium, copper, iron, magnesium, manganese, nickel, phosphorus, potassium, silicon, sodium, strontium, titanium, and zinc (Table 3). In addition to these elements, whole barley seeds contained molybdenum and silver which were not found in the leachates.

The amount of elements lost from the seeds increased with increasing time of soaking from 12 to 20 hours. However, less amounts of substances were found at 24 hours of soaking than at 20 hours (Table 3).

B. Continuous and fractionated leachates

Leachates from continuous soaking of seeds under anaerobic conditions at 20° C. for 6, 12, 18 and 24 hours were compared with fractionated leachates obtained by using one lot of 1,000 seeds but changing the water every six hours for a total of 24 hours. Ninhydrin positive substances in these leachates were analyzed as previously described (TROLL and CANNAN, 1953).

A comparison of continous and fractionated leachates revealed that most of the ninhydrin positive substances were leached within six hours of seed soaking. Subsequent leachates from the same seeds showed a very small amount of loss. For instance, the amounts of ninhydrin positive substances obtained after four successive leachings of 6 hours each were 90.4, 8.8, 0.6 and 0.2 per cent of total respectively.

C. Leachates from embryos and endosperms

Embryos were separated from endosperms of the seeds that had been stored in a humid chamber for 5 days. Since no water came in direct contact with the seeds it was assumed that nothing was leached from the seeds while in humid storage. Leachates from embryos, endosperms and whole seeds were obtained and studied for the different substances already described.

More than twice as much total amino acids were leached from isolated embryos and endosperms as from the whole seed. Endosperm leachates contained more total ninhydrin positive substances than embryo or whole seed leachate. Thus, ninhydrin values in terms of leucine equivalents (L-E) per endosperm, embryo and whole seed were .171, .214

and .150 μm respectively. On the other hand, 13 to 18 times greater values were obtained for embryos (53.64) than for endosperms (4.15) or whole seeds (3.05), when expressed in terms of L-E per 100 mg. of seed tissue. Semi-quantitative estimations of amino acids yielded greater values for leachates from isolated embryos and endosperms than from whole seeds.

Chromatograms for sugars showed the presence of sucrose, raffinose, fructose and glucose in all the embryo and endosperm leachates. However, no sucrose or raffinose was obtained from whole seed. Embryo and endosperm leachates indicated the presence of 3 distinct organic acids on paper chromatograms. On the other hand, leachates from whole seed showed only two organic acid spots. Paper chromatograms showed the presence of inorganic orthophosphate in addition to one sugar phosphate for whole seed leachates and 1 to 2 sugar phosphates for various embryo and endosperm leachates. No other UV absorbing compounds were found in the embryo and endosperm leachates.

D. Effect of osmotic concentration of soaking solution on leaching process

Seeds were soaked in several concentrations of glucose and potassium chloride (KCl) solutions. Leachates obtained in this manner were analyzed for total ninhydrin positive substances.

TABLE 4. *Photometric determinations of total ninhydrin positive substances in barley seeds leached in various concentrations of KCl solutions for 6 hours at 20° C. in nitrogen*

Concentration of KCl	Optical density at 570 mμ	Leucine equivalent μm/ml
Saturated	0.057	.022
4 Molar	0.058	.023
3 „	0.066	.027
2 „	0.107	.047
1 „	0.165	.075
.1 „	0.447	.211
.01 „	0.422	.199
.001 „	0.389	.186
.0001 „	0.456	.215
Water	0.488	.231

The amounts of ninhydrin positive substances leached out of the seeds varied with the osmotic concentration of the soaking solution. Ninhydrin color values decreased with increasing concentration of the potassium chloride soaking solution (Table 4). Similar results were obtained with glucose soaking solutions.

E. Leachates from irradiated and pressure stored seeds

Seeds were either exposed to 30 Kr of X-ray, stored for 6 weeks at 100 pounds per square inch of oxygen or nitrogen, or x-rayed and stored before leaching. An additional leachate was obtained from 1,000 seeds exposed to 140 Kr of X-rays. These leachates were studied for various substances as already described.

No difference in the amounts of amino acids was found in leachates from irradiated (30 and 140 Kr), stored (nitrogen and oxygen pressure 100 lbs/sq.in. for six weeks) and irradiated+stored seeds. Paper chromatograms showed the presence of glucose and fructose in all the leachates from x-rayed, non-x-rayed, stored and non-stored seeds.

No general trends were observed in leachates from irradiated and pressure stored seeds. However, some specific examples of increased amounts of inorganic elements (aluminum and iron) in the leachates were found with increased seed damage owing to X-radiation and pressure storage. For example, concentration of iron in nitrogen leachings of 30 Kr, pressure stored, stored+30 Kr, and 140 Kr x-irradiated seeds was 500, 600, 2,500 and 1,000 ppm. of ash respectively.

IV. DISCUSSION

An analysis of the water (pH 5.8) in which barley seeds were soaked (leachates) showed the loss of many biologically important water soluble substances from the seeds. Included were many nitrogenous compounds (ninhydrin positive) among them 14 to 16 amino acids, an amino acid amide and sugars, sugar phosphates, organic acid, an organic ester, many inorganic elements and possibly other unknown compounds from the seeds. Loss of amino acids was indicated after only 15 minutes of seed soaking. Hence, even a slight amount of soaking apparently changed the chemical composition of the seeds.

The role of energy-rich compounds like ATP and protein synthesis in chromosome rejoining process has been discussed by WOLFF (1957, 1958) and KIHLMAN *et al.* (1957). Sugars, sugar phosphates and amino

acids which are free in the seed and are readily available sources of energy and many inorganic elements may be very important in the repair of radiation damage and may even protect seeds to some extent by acting as absorbers of radiation energy. Thus, it follows that increased X-ray susceptibility of soaked seeds reported in another paper (KAMRA *et al.*, 1960) may be due in part to the loss of these compounds by soaking.

Amounts of amino acids and inorganic elements leached increased with increased time of soaking. Ninhydrin positive substances reached a maximum in six hour leachates and then disappeared steadily when soaking was extended beyond six hours. Greater quantities of total ninhydrin positive substances were leached in leachates made in the presence of nitrogen than in the presence of oxygen. On the other hand, inorganic elements reached a maximum after 20 hours and then appeared to be partially reabsorbed. Other substances not tested may have been lost in the leachates.

An analysis of continuous and fractionated leachates demonstrated that ninhydrin positive substances including amino acids, occurred free inside the seeds and were not due to progressive protein degradation. Thus, 90 per cent of the ninhydrin positive substances were recovered within 6 hours of leaching, 9 per cent in the next 6 hours and less than one per cent in subsequent soakings up to 24 hours.

It should be mentioned here that the 1956 seeds used in experiments were possibly somewhat immature. Ninhydrin tests made later on leachates from fully mature seed (1958) crop gave one-half of the 1956 crop values. In the 1958 seed crop, although 90 per cent of the amino acids were leached within 6 hours, they did not disappear after 6 hours as in the 1956 seed experiment. Their concentration increased slightly up to 18 hours and then stayed constant.

Amounts of nitrogenous compounds lost from isolated embryos and endosperms were twice that leached from intact seeds. This, in part, may be explained by the presence of the more exposed surface in isolated embryos and endosperms than in intact grains. However, not all of this increase is due to injury to embryos and endosperms. For example, no sucrose or raffinose was leached from intact seeds but both were found in the embryo and endosperm leachates (Fig. 2). Furthermore, more spots for organic acids and sugar phosphates were detected on paper chromatograms from embryo and endosperm leachates than from intact seed leachates. Thus, it would seem that the seed coat covering (pericarp and testa) of barley is differentially permeable. The loss of certain

compounds from the seeds (sucrose, raffinose, certain organic acids and sugar phosphates) is prevented, whereas other compounds (many amino acids, glucose, fructose, certain organic acids, sugar phosphates and many inorganic elements) are allowed to pass into the medium.

The quantity of ninhydrin positive substances lost from seeds decreased with increasing concentration of the soaking solution. This was shown by soaking seeds in a series of concentrations of glucose and potassium chloride solutions. Thus, the osmotic concentration of the solution used for seed soaking has a definite influence on the amount of ninhydrin positive substances leached from the seeds.

All of the organic compounds tested from leachates of x-rayed (30 Kr), pressure stored (100 lbs. per square inch of oxygen or nitrogen pressure for 6 weeks) and irradiated+stored seeds, were of the same type and quantities as were obtained in non-treated seed leachates. However, specific increases in the amounts of inorganic constituents leached were noticed in irradiated and pressure stored seed leachates. Specifically, iron and aluminum are two of the metallic constituents which seem to be related to the x-ray induced damage.

SUMMARY

The water in which x-rayed and control barley seeds were soaked (leachates) was analysed in order to relate the substances in the leachates to changes in the radiosensitivity of seeds. Leached substances included several nitrogen compounds (ninhydrin positive). Among them were several amino acids (α-alanine, γ-amino butyric acid, arginine, asparagine, aspartic acid, glutamic acid, glycine, leucine, isoleucine, methionine sulphoxide, proline, serine, threonine, valine and probably pipecolinic acid), sugars (glucose and fructose), one or two sugar phosphates, organic acids and many inorganic elements (aluminum, barium, calcium, chromium, copper, iron, magnesium, manganese, nickel, phosphorus, potassium, silicon, sodium, strontium, titanium, and zinc). In addition to all of the above mentioned inorganic elements, whole seeds contained some molybdenum and silver. Seed leachates failed to show the presence of any fatty acids and purine or pyrimidine compounds.

Loss of amino acids and other ninhydrin positive substances was indicated within 15 minutes of seed soaking and was more in presence of nitrogen than in oxygen. Hence, even a slight amount of soaking appeared to change the chemical nature of the seeds. Most of the nin-

hydrin positive substances leached within 6 hours of soaking; with additional soaking up to 24 hours they increased somewhat in fully mature seeds (1958) or disappeared steadily if the seeds were a little immature (1956). Twice the quantity of substances leached out of immature seeds as out of fully mature seeds. On the other hand, the concentration of leached inorganic elements increased with time of soaking and reached a maximum at 20 hours. Further soaking caused their reabsorption. Soaking may also involve other substances not tested in the leachates.

Analysis of continuous and fractionated leachates indicated that amino acid leached from the seeds occur free inside the seeds and are not due to protein degradation.

Evidence obtained indicated that the seed coat covering is differentially permeable. Twice the concentration of ninhydrin positive substances was detected in embryo+endosperm leachates as in whole seed leachates. Moreover, many additional substances, including sucrose and raffinose, were detected in embryo and endosperm leachates.

The concentration of the solution used for seed soaking had a definite influence on the amount of ninhydrin positive substances leached from the seeds. This was shown by soaking seeds in a series of concentrated glucose and potassium chloride solutions. X-radiation and pressure storage (100 lbs. per square inch of oxygen or nitrogen for six weeks) of seeds did not seem to affect the types and amounts of organic substances lost from the seeds. However, inorganic substances, specifically iron and aluminum, were lost and later reabsorbed. This reabsorption seemed to coincide with the apparent recovery in x-ray induced damage to barley seeds.

The x-ray response of leached barley seeds is reported in another paper (KAMRA *et al.*, 1960).

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SPECIES CROSSES WITHIN THE GENUS BRASSICA

I. ARTIFICIAL BRASSICA JUNCEA COSS

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I. INTRODUCTION

THE formation of species and the interrelations between species are central problems of biology which have been made accessible to more intensive research through the development of cytogenetics. Already at the beginning of the present century, ROSENBERG (1909) showed that the hybrid between *Drosera longifolia* ($n=20$) and *D. rotundifolia* ($n=10$) formed $10_{II}+10_{I}$ at meiosis. Cytological studies of meiosis, carried out during the 1920's and the 1930's on a large number of species hybrids, often made it possible to judge the relations between the species involved (TÄCKHOLM, 1922; KIHARA, 1924; GOODSPEED and CLAUSEN, 1928). In many cases species hybrids have resulted in new, constant forms, which must be considered as new species. As examples it may suffice to mention *Nicotiana digluta* (CLAUSEN and GOODSPEED, 1925) and *Raphanobrassica* (KARPECHENKO, 1927, 1928). MÜNTZING's (1930 a and b, 1932) synthesis of *Galeopsis tetrahit* presented the first artificial synthesis of a species already known in its wild stage.

At about the same time intensive work was carried out in Japan on species within the genus *Brassica* (MORINAGA, 1928, 1929 a, b, c, 1931, 1933, 1934; SASAOKA, 1930). Based on studies of chromosome pairing in species hybrids MORINAGA (1934) suggested that the high chromosome species *Brassica carinata* BRAUN, *B. juncea* COSS. and *B. napus* L. were derived from the low chromosome species *B. nigra* KOCH, *B. oleracea* L. and *B. campestris* L. as amphidiploid hybrids (Fig. 1).

The correctness of this hypothesis has since been proved by the synthesis of *B. napus* from crosses between *B. campestris* and *B. oleracea* (U, 1935; FRANDSEN, 1947; RUDORF, 1950, 1958; KOCH-PETERS, 1953; OLSSON, 1953; OLSSON *et al.*, 1955; HOFFMAN and PETERS, 1958) of

B. juncea from crosses between *B. campestris* and *B. nigra* (FRANDSEN, 1943; RAMANUJAM and SRINIVASACHAR, 1943; OLSSON, 1947) and of *B. carinata* from crosses between *B. oleracea* and *B. nigra* (FRANDSEN, 1947; MIZUSHIMA, 1950; MIZUSHIMA and KATSUO, 1953; OLSSON, unpubl.).

The aim of the present investigation has been to make a close study of artificial *B. juncea*, in order to elucidate its relations to the natural species and to evaluate its practical potentialities.

II. MATERIAL AND METHODS

In crosses planned for the purpose of synthesizing *B. juncea* COSS. (brown mustard), three varieties of *B. campestris* L. ssp. *oleifera* (METZG.) SINSK. (turnip rape, cf. OLSSON, 1954) were used (Mette's, Hunsrücker's and Strube's summer turnip rape). The varieties are morphologically rather alike, but Strube's summer turnip rape is the most valuable one from a practical point of view. Of *B. nigra* KOCH (black mustard), two varieties were used which were obtained from the Weibullsholm plant breeding station. One of these is low growing with low branches and fairly long pods, the other one is taller with high branches and fairly short pods. For comparison, natural *B. juncea* obtained from Brno, Stalingrad and Sophia was mainly used, but the artificial strains were also compared with several other varieties of natural brown mustard, tested in trials at the Swedish Seed Association (ANDERSSON and OLSSON, 1953). An artificial *juncea* obtained from FRANDSEN (1943) was also tested in field trials. Important morphological characters of the different species will be discussed below in connection with the description of artificial *B. juncea*.

In order to perform the cross between turnip rape and black mustard also on the tetraploid level, chromosome doubled types were developed by colchicine treatment. Seedlings in the cotyledon stage were treated with colchicine. In 1943 and 1944 a mixture was used consisting of 2 parts 2 per cent colchicine solution and 1 part 3 per cent agar solution. Later, $^1/_2$—1 per cent solutions of colchicine without agar were used. The characteristics of the tetraploids will be discussed in a special section

The crosses were mainly performed on material growing in the field. Buds in the right stage were emasculated, enclosed in pergamyn bags, pollinated after about 36 hours and then again enclosed in bags. Crosses between cruciferous plants are as a rule fairly easily made, but species crosses sometimes give rise to certain complications so that the percen-

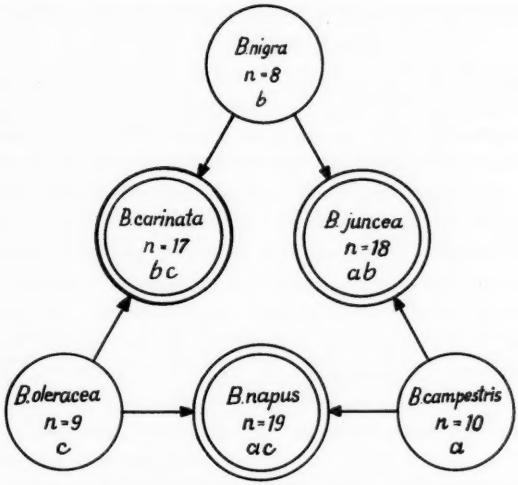


Fig. 1. The derivation of the high chromosome *Brassica* species according to U. (From FRANDSEN 1943.)

tages of pod and seed setting observed may be rather misleading. The pods from such crosses are often well developed without containing any normal seeds. In addition most of the seeds obtained from crosses between *B. campestris* and *B. nigra* are not true hybrid seeds but develop into plants identical with the mother plant. U (1935) regards such plants as the result of parthenogenetic development of eggs stimulated by the foreign pollen, and followed at an early stage by a doubling of the chromosomes. NISHYAMA and INAMORI (1953) consider that "they could have originated from pseudogamy or more probably from haploid parthenogenesis followed by doubling its chromosome number". According to FUKUSHIMA (1931), there are also PMC's with double the chromosome number; the experiences from the crosses to be described here confirm the occurrence of unreduced gametes. As the so-called matromorphous or matroclinous plants seem to be more common in interspecific than in intraspecific crosses, it seems probable that seeds may be formed without fertilization. On the other hand, it should be pointed out that such seeds may simply be the result of some failure in the crossing technique. There is always the chance that pollen from the mother variety, floating in the air, may land on the pistil when the bag is opened for pollination.

The frequencies of well-developed and stained pollen were determined in a mixture of aceto-carmine and glycerine (1 : 1). The percentage of morphologically well-developed pollen grains is taken here as pollen fertility, and the number of well-developed seeds in per cent of the ovules in the ripe pods is taken as seed fertility. The true pollen and seed fertilities are probably somewhat lower than the figures thus computed. All morphologically well-developed pollen grains do not germinate and this is probably especially the case in semisterile or highly sterile material. The number of ovules per pod will be estimated at a slightly too low figure, since it is sometimes difficult to recognize the scars from all ovules; this again is especially the case in highly sterile plants with shrunken pods. The percentage of well-developed seeds will thus be computed on the basis of a somewhat too low total number of ovules and the percentage values will be a little too high. Comparisons between fertilities of different materials will thus be approximative, especially if one of the combinations has a low fertility, and thus the differences observed are minimum values.

Somatic chromosome numbers were determined in root-tips fixed in MÜNTZING's (1933 a, p. 131) modification of NAVASHINS's fixative and stained with crystal violet. Flower buds were fixed, either in acetic alcohol (1 : 3) alone or, usually, the acetic alcohol fixation was followed by fixation in Navashin—Müntzing, Benda or La Cour BD (DARLINGTON—LA COUR, 1942). It was difficult to bleach the Benda fixations before staining, and this method did not give better results than the acetic alcohol — Navashin fixations. These latter fixations were treated for about 14 hours with 1 per cent chromic acid before staining with crystal violet.

When a species is synthesized by experimental methods, the resulting material is sometimes called "artificial" and sometimes "synthetic", and no fixed rules are established for the use of these terms. As an example it may be mentioned that when MÜNTZING first presented his synthesis of an already existing species, he called his new material "artificial" *Galeopsis tetrahit* (MÜNTZING, 1930 b), later to change it to "synthetic" (MÜNTZING, 1932). In earlier papers, the present author (OLSSON, 1953; OLSSON *et al.*, 1955) used the term "artificial" and for this reason he chose to use the same term for the synthesized *B. juncea* material described in this paper.

The amphidiploid hybrid has been designated as A_1, A_2 etc., the figures denoting the generation. The number of individuals are denoted n, mean $\bar{x}$ and standard error m.

III. SOME DATA ON AUTOTETRAPLOIDS OF BRASSICA NIGRA AND B. CAMPESTRIS

For the analysis of the artificial material of *B. juncea*, it is of interest to have some data concerning the artificial autotetraploids of *B. nigra* and *B. campestris* which have been used as parents in synthesizing *juncea*. Consequently, a brief summary of data is given here concerning these autotetraploids and also concerning autotetraploids of *B. juncea*.

As expected the autotetraploids are characterized by an increase in cell size. The length of the stomatal cells in 2x and 4x black mustard was $27.2 \pm 0.31\ \mu$ and $38.9 \pm 1.03\ \mu$, respectively, and in 2x and 4x summer turnip rape $26.4 \pm 0.40\ \mu$ and $39.1 \pm 0.99\ \mu$, respectively. The diameter of pollen grains in Belling-glycerine was about 25 per cent larger in the tetraploids than in the mother strains (Table 1). The increase in cell size results *i. a.* in thicker leaves and in larger buds, flowers and seeds.

The autotetraploids of *nigra* and *campestris* were vegetatively well developed and in our trials they were taller than the corresponding diploids. The autotetraploid *juncea*, on the other hand, was markedly weaker and lower than the diploid (Table 1); and it was evident that the 72-chromosome variety of *juncea* exceeded the optimal chromosome number of the species.

The pollen fertility of tetraploid *nigra* was as high as in the diploid. In tetraploid *campestris*, however, there was some decrease; and in tetraploid *juncea* a considerable decrease in pollen fertility was noted. The determination of seed fertility, number of well-developed seeds per pod and seed yield is complicated by the fact that the tetraploid is easily crossed with the diploid, resulting in triploid embryos which abort to a great extent (*cf.* OLSSON and RUFELT, 1948). The tetraploid material used for these determinations must be separated from the diploid material, which results in increased risks for soil differences and for irregular and often severe attacks of insect pests. Usually, therefore, the seed fertility was not determined in the autotetraploids, and data were available only for a material of *B. nigra*. In this material the diploid had 84 per cent and the tetraploid 42 per cent well-developed seeds.

The number of ovules per pod was approximately the same in diploids and in tetraploids, and the numbers of well-developed seeds per pod, quoted in Table 1, gave a fair estimate of the differences between diploids and tetraploids in regard to seed fertility and zygotic lethality. According to results so far obtained, the seed yield of the tetraploids was lower than that of the diploids, and this was especially marked in *B. juncea*. The oil content of the tetraploids was also considerably lower than that of the diploids, whereas the content of crude protein was higher in the tetraploids (OLSSON, 1960).

The diploid *B. campestris* and *B. nigra* were as a rule self-incompatible and the tetraploidy did not seem to result in any increase in self-compatibility (HOWARD, 1942 a; OLSSON, 1953; BATEMAN, 1955).

As a rule, the meiosis of the diploid *B. nigra* and *B. campestris* was very regular, whereas there was a certain amount of quadrivalent formation in the autotetraploids. In tetraploid *nigra*, RAMANUJAM and DESHMUKH (1945) found on an average 0.8 quadrivalents per cell and the regular chromosome number (16+16) appeared in 75 per cent of the cells in second metaphase. In the material of tetraploid *nigra* studied here one or two ring or rod quadrivalents appeared fairly often at M_I, but 16_{II} was by far the most common configuration. About $^3/_4$ of the M_{II} plates

TABLE 1. *Comparison between diploid and tetraploid forms of Brassica nigra, B. campestris and B. juncea.*

Species	Ploidy	Chrom. number	Plant height			Pollen diameter			Pollen fertility		Number of good seeds per pod			Seed weight per plant			1000 grain weight		
			n	Height cm	Rel. val.	n	μ	Rel. val.	n	Per cent good pollen	n	Number	Rel. val.	n	Gram	Rel. val.	n	Gram	Rel. val.
B. nigra, low	2x	16	97	108.5±1.17	100	120	26.7±0.31	100	150	96.7	147	9.66±0.12	100	97	4.10±0.34	100	10	1.66±0.073	100
				t=4.86***											t=1.49°				
„ „	4x	32	80	117.1±1.33	108	134	33.5±0.42	125	146	97.0	130	4.91±0.15	51	80	3.39±0.33	83	10	2.98±0.135	180
B. campestris	2x	20	98	94.0±1.28	100	75	26.2±0.16	100	125	94.3	148	17.83±0.41	100	98	6.27±0.59	100	25	2.80±0.070	100
				t=4.78***											t=2.05*				
„	4x	40	99	101.6±0.94	108	73	32.9±0.22	126	124	88.6	149	13.46±0.35	75	99	4.71±0.48	75	25	3.70±0.089	132
B. juncea	2x	36	50	139.3±1.26	100	50	27.4±0.15	100	100	95.5	50	15.6 ±0.38	100	50	12.3 ±0.90	100	25	3.06±0.073	100
„	4x	72	50	113.9±2.21	82	50	34.6±0.27	126	100	71.3 [1]	50	4.9 ±0.24	31	50	1.2 ±0.04	10	25	3.81±0.090	125

[1] The 1950 values are remarkably low.

TABLE 2. *Results of crosses between B. campestris and B. nigra.*

Combination	Number of pollinated flowers	Number of seeds harvested and sown	Number of F_1-plants	Number of matromorphous plants
2x *B. campestris*×2x *B. nigra*	883	192	11	136
Reciprocal	785	76	0	65
4x *B. campestris*×4x *B. nigra*	1772	111	40	59
Reciprocal	1153	43	0	38

studied had 16 chromosomes. RAMANUJAM and DESHMUKH (1945), also studying meiosis of tetraploid *B. campestris*, report a mean number of 1.9 quadrivalents per cell and M_{II} plates with 20 chromosomes in 81 per cent of the cells studied.

IV. B. CAMPESTRIS L.×B. NIGRA KOCH. ARTIFICIAL B. JUNCEA COSS

1. Synthesis

SINSKAIA (1927) and FRANDSEN (1943) failed in their attempts to cross diploid *B. campestris* and *nigra*. Such a hybrid is mentioned, however, by U, MIZUSHIMA and SAITO (1937). By pollinating 1513 flowers of *campestris* with pollen from *nigra*, RAMANUJAM and SRINIVASACHAR (1943) obtained 65 hybrid plants and several matromorphous ones. The reciprocal cross gave no hybrids. The present author also obtained hybrids only when *campestris* was used as mother; in all 883 pollinated flowers gave rise to 11 F_1-plants and many matromorphous offspring (Table 2).

The synthesis of *B. juncea* can be effected either by colchicine doubling of the 18 chromosome hybrid (RAMANUJAM and SRINIVASACHAR, 1943) or by crossing autotetraploids of *B. campestris* and *B. nigra* (HOWARD, 1942; FRANDSEN, 1943). As seen from Table 2, in the present experiments, 1772 flowers of tetraploid *campestris*, pollinated with pollen from tetraploid *nigra*, gave 40 hybrid plants. The reciprocal cross gave no hybrid seeds; the combination is possible, however, and 4 out of the 13 hybrid seeds of FRANDSEN (1943) having been harvested on tetraploid *nigra*.

The results of the cross are dependent not only on the skill of the worker and other environmental conditions, but also on the genotype and on the physiology of the plants used. It was observed that if two parent plants were capable of producing F_1 seed at all, they often gave

several seeds. Consequently, the hybrids obtained descended from a fairly small number of parent individuals. For example, the 6 hybrids obtained in diploid crosses in 1951 were all derived from one individual cross combination. It is difficult to judge whether crosses between tetraploids have a greater chance to success than crosses between diploids. The values in Table 2 do not indicate a significant difference ($\chi^2=3.09$).

Seeds developing into true hybrids were as a rule smaller than those developing into matromorphous plants, or those obtained from crosses within the variety.

As already mentioned, diploid *B. campestris* has $2n=20$ and diploid *B. nigra* $2n=16$. Of the 11 hybrid plants derived from crosses between the diploid species, 9 had 18 chromosomes whereas one plant had 28 and one 36 chromosomes. The plant with 28 chromosomes had two genomes of *B. campestris* and one of *B. nigra*, and was evidently derived from an unreduced egg and a reduced male gamete. The plant with 36 chromosomes may be either the result of two unreduced gametes or of a somatic doubling at an early stage in the development of the zygote. This plant is of special interest as it shows that *B. juncea*, the amphidiploid of *B. campestris* and *B. nigra*, may derive directly from a cross

TABLE 3. *Survey of A_1 plants of artificial B. juncea.*

Artificial *B. juncea*, number	Cross combination	A_1 material, year and field number
1—5	4x Mette's summer turnip rape × 4x Weibull's black mustard (high)	1945—219—1
6—21	4x Hunsrücker's summer turnip rape × 4x Weibull's black mustard (high)	„ —220—1, 2, 3
22	4x Strube's summer turnip rape × 4x Weibull's black mustard (high)	„ —221—1
23	2x Strube's summer turnip rape × 2x Weibull's black mustard (low)	1949—406—7
24	(2x Strube's summer turnip rape × 2x Weibull's black mustard (low) × 2x Strube's summer turnip rape [1])	„ —414—7
25—35	4x Strube's summer turnip rape × 4x Weibull's black mustard (low)	1953—474
36—42	4x Gruber's winter turnip rape × 4x Weibull's black mustard (low)	1956—316

[1] The primary plant had 18 chromosomes.

Fig. 2. *B. campestris*, 28 and 18 chromosome hybrids between *B. campestris* and *B. nigra*, and *B. nigra*.

between the diploid parent species. This plant will be referred to as a.j. 23.

Of the 40 hybrid plants derived from crosses between tetraploids, 33 had probably 2n=36, 4 had possibly 37 and 2 plants (a.j. 33 and 35) had 38—40 chromosomes. A_1 plants with aberrant chromosome numbers are to be expected as a consequence of the disturbed meiosis of the autotetraploid parents and have been recorded among others by FRANDSEN (1947) in artificial rape.

In order to simplify the further treatment of the material, Table 3 gives a survey of the artificial *B. juncea* plants obtained, these plants being numbered in a consecutive series. The A_1 material of 1945 (a.j. 1—22) flowered in a group and crosses were made between different plants in order to obtain as much seed as possible. The same was true of a.j. 25—35 and a.j. 36—42, respectively. A.j. 23 and 24 derive from diploid parents, but whereas a.j. 23 descend from an F_1 with 36 chromosomes, a.j. 24 descends from an F_1 plant with 18 chromosomes. A.j. 23 and 24 were propagated separately from each other and from the other material.

The measurements of cell size and the morphological details presented

here are based on a.j. 1—32 and 34. A.j. 33 and 35, which have a definitely aberrant chromosome number and are also morphologically distinctly different from the other hybrids, are not included in the following treatment of the material. The cross between the winter type of *B. campestris* and *B. nigra* (a.j. 36—42) was made in an attempt to produce a winter type of *B. juncea*. This material was not subject to the same selection for fertility as the remainder and is here only briefly reported.

2. Morphology

The 18 and 36 chromosome hybrids were almost intermediate between the parents and at the same time similar to the natural *B. juncea*. As expected, the 18 chromosome hybrids were more slender than normal brown mustard and had smaller buds and flowers. In addition, the low fertility of these hybrids resulted in poorly developed anthers and pods. The three hybrids with 28 and 38 chromosomes resembled *B. campestris* more than the former ones and may be said to be intermediate between

Fig. 3. *B. campestris* (left), *B. nigra* (right) and the amphidiploid hybrid (middle).

Fig. 4. Natural (left) and artificial *B. juncea* (right).

this species and *B. juncea*. This was especially evident in the increased degree of clasping of the upper leaves (Fig. 2).

Both *B. campestris* and *B. nigra* are marked cross-fertilizers and, since unselected plants from normal populations were used for the crosses, there were considerable differences between different A_1 plants of artificial *B. juncea*. They were highly heterozygous and, since they were usually cross-pollinated, subsequent generations exhibited great variation. This variation was further accentuated by abnormal meiosis in A_1. A completely fertile and selfed A_1 plant (a.j. 28) gave, however, a rather homogenous progeny.

For a better comparison between artificial *B. juncea*, the parents and natural *juncea*, some important morphological characters will be treated in greater detail. Both parent species as well as natural and artificial *juncea* are characterized by strongly lobed rosette leaves with crenate-dentate margins. In all of them the shape of the leaves changes towards the upper part of the plant, where they are undivided and with entire margins. At the same time, there are considerable differences between the species. The middle and upper leaves of *B. campestris* are strongly

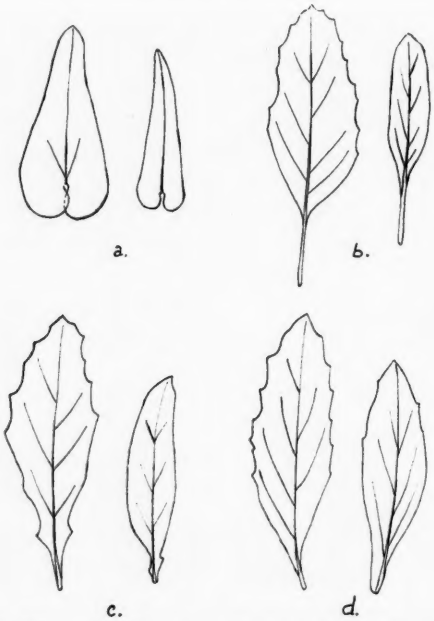


Fig. 5. Upper stem leaves. a: *B. campestris*; b: *B. nigra*; c: artificial and d: natural *B. juncea.*

clasping, undivided and, at least in the upper part, with an entire margin, whereas even the upper leaves of *B. nigra* have a distinct, fairly long and somewhat weak petiole and lobed leaves with crenate-dentate margins occuring much higher up than in *B. campestris*. In *B. juncea*, the upper leaves are lanceolate with the base gradually narrowing into the short petiole, only weakly lobed or — in the uppermost part — entire. In artificial *juncea*, the upper leaves are sometimes a little longer than in the natural forms studied, while the agreement is very good in regard to leaf shape and length of petiole (Fig. 5). In regard to lobation, hairiness and colour of the lower leaves, artificial *juncea* is intermediate between the parents and in good agreement with the natural species.

In the bud stage, artificial brown mustard is very different from black mustard (Fig. 6). In the latter, the buds have very short pedicels and are densely crowded, whereas in the former, as well as in the natural material, the buds are clearly pedicellate. *B. campestris*, on the other hand, has still longer pedicels. During flowering, the flowers stand

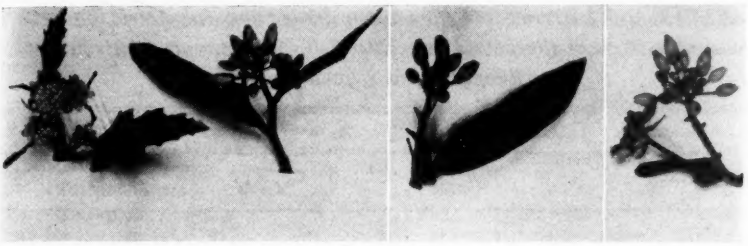


Fig. 6. Flower buds of *B. nigra*, artificial and natural *B. juncea* and *B. campestris*.

higher than the buds in *campestris* whereas in *nigra* the buds are clearly visible above the flowers. Artificial as well as natural *juncea* is intermediate in this respect with flowers and buds at about the same level.

The length of the pedicel was measured in some diploid and tetraploid populations of the parents and in some populations of artificial and natural *juncea*. As seen from Table 4, there were no significant differences between artificial and natural *juncea*, which again were intermediate between and significantly different from the parents.

The general appearance of the flowers of the artificial *juncea* is similar to that of the natural forms of the species. The sepals are longer than in *campestris* and broader than in *nigra*. The petals of *Brassica* consist of a broad, oval upper part and a narrow, claw-like lower part, standing at about 90° to each other. There are differences between the species studied here in regard to the relative length of the two parts of the petal. Measurements were made on A_1 plants in 1945 and 1953 and

TABLE 4. *Length of flower pedicel in mm in B. campestris, B. nigra and artificial and natural B. juncea.*

No.	Material	n	$\bar{x} \pm m$	t	for no.
1	Diploid *B. nigra*	35	3.8±0.11		
2	Tetraploid *B. nigra*	35	4.7±0.17	7.22***	2—4
3	*B. juncea* from Brno	35	7.4±0.35	0.84°	3—4
4	Artificial *B. juncea*	35	7.1±0.27	8.77***	4—5
5	Tetraploid *B. campestris*	35	11.2±0.38		
6	Diploid *B. campestris*	35	11.9±0.51		
7	*B. juncea* from Hungary	25	8.1±0.23		
8	*B. juncea* from Sophia	25	8.6±0.31		
9	Artificial *B. juncea*×*B. juncea* from Brno	25	6.2±0.24		
10	FRANDSEN's artificial *B. juncea*	25	8.1±0.27		

TABLE 5. *The relation between the lengths of the broad and the narrow parts of the petals in artificial B. juncea, natural B. juncea, B. nigra and B. campestris.*

Material	A_1 material of artificial *B. juncea*		A_3—A_4 material of artificial *B. juncea*	
	n	x ± m	n	$\bar{x}$ ± m
Diploid *B. nigra*			35	0.92±0.017
Tetraploid *B. nigra*	30	1.02±0.022	35	1.03±0.014
B. juncea from Brno	30	1.32±0.022	35	1.32±0.024
Artificial *B. juncea*	30	1.33±0.026	35	1.33±0.015
Tetraploid *B. campestris*	30	1.74±0.026	35	1.85±0.035
Diploid *B. campestris*			35	1.83±0.032
B. juncea from Hungary			25	1.41±0.026
B. juncea from Sophia			25	1.44±0.018
Artificial *B. juncea*×*B. juncea* from Brno			25	1.32±0.020
FRANDSEN's artificial *B. juncea*			25	1.41±0.024

on natural *juncea* as well as on the tetraploid parents in the same years, further on A_3—A_4 material descending from the A_1 material of 1945 and a corresponding material of natural *juncea* and of the parents. On the A_1 material, five petals from each plant were measured in 1945 and three in 1953, whereas only two petals per plant were measured in the A_3—A_4 material. As seen from Table 5, both A_1 and A_3—A_4 are intermediate between the parents and in agreement with natural *juncea* in regard to the relation between the two parts of the petal.

In *B. nigra*, the difference in length between the long and the short stamens is less than in *B. campestris, B. juncea* being intermediate between the parents. As seen from Table 6, however, there is a fairly significant difference in this respect between the artificial *juncea* and the natural material studied.

The arrangement and general appearance of the pods are of great interest because these characters are given considerable weight in the taxonomy of the genus *Brassica.* The pods of *campestris* are long and borne on long pedicels standing more or less at a right angle to the stem; the beak is long and gradually narrowing. In *nigra*, the pods have short pedicels and stand parallel to the stem; they are short with a square cross section and have a short thin beak.

In natural as well as in artificial *B. juncea*, the arrangement of the pods and their general type are intermediate between the parents

TABLE 6. *The relation between the lengths of the long and the short stamens in A_3—A_4 material of artificial B. juncea, natural B. juncea, B. nigra and B. campestris.*

No.	Material	n	$\bar{x} \pm m$	t for no.
1	Diploid *B. nigra*	35	1.41±0.025	1.60° 1—3
2	Tetraploid *B. nigra*	35	1.30±0.013	7.21*** 2—4
3	*B. juncea* from Brno	35	1.47±0.028	2.82** 3—4
4	Artificial *B. juncea*	35	1.61±0.041	3.08** 4—5
5	Tetraploid *B. campestris*	35	1.80±0.046	
6	Diploid *B. campestris*	35	1.88±0.050	
7	*B. juncea* from Hungary	25	1.41±0.015	
8	*B. juncea* from Sophia	25	1.44±0.022	
9	Artificial *B. juncea*×*B. juncea* from Brno	25	1.64±0.044	
10	FRANDSEN's artificial *B. juncea*	25	1.66±0.033	

(Fig. 7). The pods stand at an angle of about 30° to the stem and, as seen from Table 7, the lengths of the pedicels, the valves and the beak are intermediate between the parents, and at the same time there are only small differences between natural and artificial *juncea*. It should be pointed out that there are considerable differences between different varieties of natural *juncea* in regard to position and size of the pods (see ANDERSSON and OLSSON, 1953, Fig. 4). Even within artificial *juncea*

TABLE 7. *Lengths of pod pedicel, valves and beak in artificial and natural B. juncea, B. nigra and B. campestris.*

Five pods measured on each of 50 plants of each variety or family.

Variety	Field no. 1948	Pod pedicel, length, mm $\bar{x} \pm m$	Valves, length, mm $\bar{x} \pm m$	Beak, length, mm $\bar{x} \pm m$
Diploid *B. nigra*	27	4.6±0.81	25.3±0.37	3.0±0.070
Tetraploid *B. nigra*	47	6.2±0.12	17.8±0.27	3.6±0.053
B. juncea from Sophia	88	12.5±0.20	29.0±0.31	7.9±0.098
B. juncea from Brno	89	12.9±0.18	31.9±0.35	9.8±0.12
Artificial *B. juncea*, A_4 family	90	11.6±0.15	29.0±0.56	8.5±0.13
" " " " "	91	10.9±0.17	30.9±0.49	9.4±0.17
" " " " "	94	13.5±0.23	31.7±0.40	8.7±0.14
" " " " "	98	10.8±0.14	34.1±0.47	10.1±0.19
Tetraploid *B. campestris*	44	18.2±0.31	47.6±0.78	23.8±0.37
Diploid *B. campestris*	24	17.4±0.35	47.3±0.84	20.7±0.47

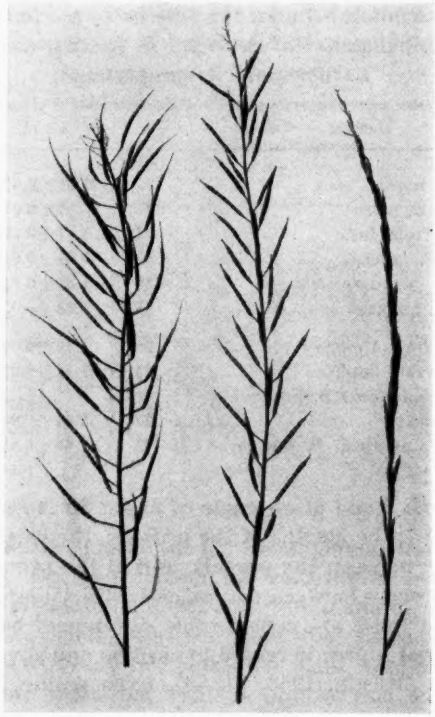


Fig. 7. Pod bearing stems of *B. campestris* (left), *B. nigra* (right) and the amphidiploid hybrid (middle).

there is a great variation in general appearance of the pods (Fig. 10), but in the artificial material no types were observed corresponding to the most extreme ones in natural material. The general appearance of the pods of the artificial types is also influenced by the often reduced fertility.

As is evident from the data given here, there is good morphological agreement between artificial and natural *B. juncea.*

3. Cell size

In order to estimate possible differences in cell size between the parent species and artificial and natural *B. juncea,* measurements were made of stomatal cells and of pollen grains. The stomatal cells were taken from the epidermis of the underside of well-developed middle leaves, the sample always being taken between the first and second side veins.

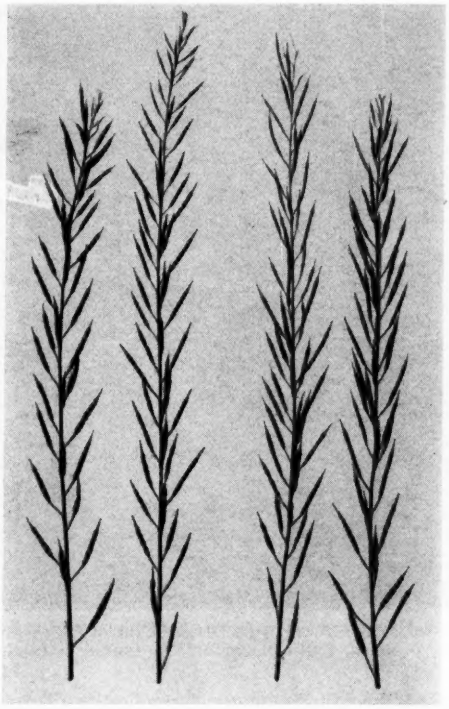
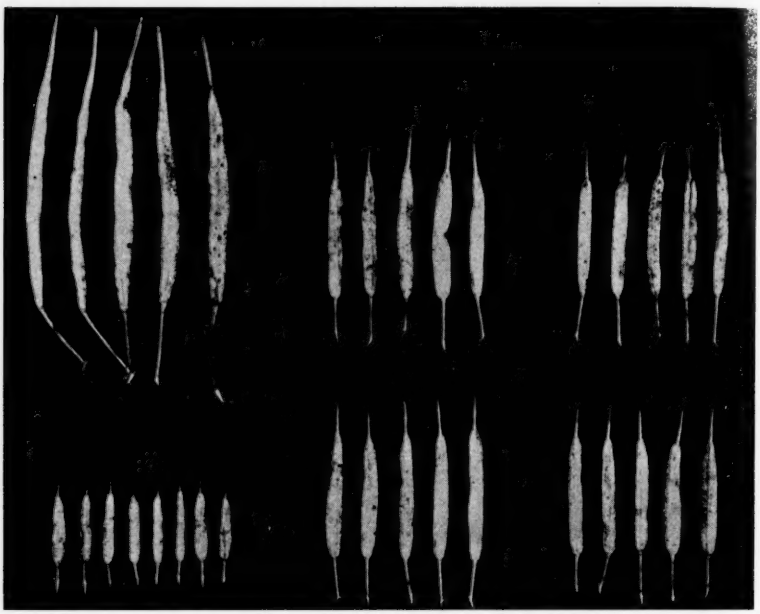


Fig. 8. Natural (left) and artificial *B. juncea* (right).

The slides were stored for some months in Belling glycerine before being measured.

In hybrid plants from crosses between diploid parents, stomatal cells of seven 18-chromosome plants had a mean length of 27.0 μ, whereas the corresponding values for one 28-chromosome plant was 29.3 and for a 36-chromosome plant (a.j. 23) 30.3 μ.

The natural *B. juncea*, although an amphidiploid, has only slightly larger stomata and pollen grains than diploid *B. nigra* and *B. campestris*. (Tables 1 and 8). On the other hand, the A_1 plants of artificial *juncea* have decidedly larger stomata than the natural types studied (in both 1945 and 1953 $P < 0.001$), and the freshly synthesized amphidiploid agrees more with the tetraploids of the parent species than with the diploids (Table 8). In comparing artificial *juncea*, which had been grown and selected for some generations, with natural material, the



F. 9. Ripe pods. Upper row: *B. campestris*, artificial and natural *B. juncea*. Lower row: *B. nigra*, artificial×natural *B. juncea*, natural *B. juncea*.

stomata of most of the families were, however, not larger than those of the natural variety with the largest cells. Thus, only a few years of cultivation tends to decrease the size of the cells in artificial *B. juncea*

TABLE 8. *The length of the stomatal cells in μ in B. nigra, natural and artificial (A_1 generation) B. juncea and in B. campestris 1945 and 1953.*

	1945		1953	
	n	$\bar{x} \pm m$	n	$\bar{x} \pm m$
Diploid *B. nigra*	10	27.2±0.31	—	—
Tetraploid *B. nigra*	10	38.9±1.03	7	33.9±2.06
Natural *B. juncea* [1]	10	28.9±0.70	11	28.7±0.60
Artificial *B. juncea*, A_1	10	36.1±1.10	9	33.7±1.08
Tetraploid *B. campestris*	10	39.1±0.99	6	35.9±0.96
Diploid *B. campestris*	10	26.4±0.40	—	—

[1] 1945 from Brno, 1953 from Stalingrad.

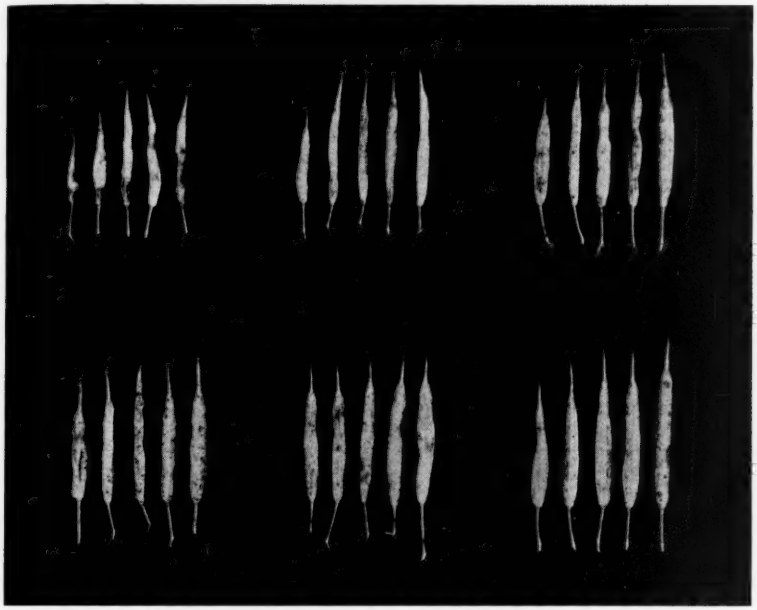


Fig. 10. Ripe pods from different A_2 plants of artificial *B. juncea*.

towards that of the natural species. This is also evident from Table 9, where a direct comparison is made between a number of A_1 plants and the families in A_3—A_8 descending from them.

It is always difficult to obtain entirely reliable measurements of the size of the stomatal cells, and these difficulties are obviously of particular importance when, as in this case, the cell size of single A_1 plants must be used to determine differences. In spite of the greatest possible care, the mean values for the individual A_1 plants — based on measurements in a.j. 6—23 of 50 cells and in a.j. 25—32 of 20 cells — were much more uncertain than the means for the different A_3—A_8 families, which were based on measurements of 20 cells in each of 25 or 50 plants. Furthermore, the different materials were grown in different years. On the other hand, brown mustard from Brno and Stalingrad, when grown together with A_1, had a cell size of 28,8 μ, and when growing together with the A_3—A_8 material a cell size of 29.3 μ. Thus, the decrease in cell size from A_1 to A_3—A_8, shown in Table 9, cannot be the results of environmental influence. Against such an hypothesis may also

TABLE 9. *The length of the stomatal cells in A_1 and in corresponding A_3—A_5 material of artificial B. juncea.*

Artificial *juncea*, number	Length of the stomatal cells in μ		Difference	t
	A_1 plants	A_3—A_5 material		
6	40.4±0.70	30.9±0.16	−9.5±0.72	13.19***
15	37.1±0.35	31.5±0.18	−5.6±0.39	14.36***
16	36.4±0.58	32.7±0.18	−3.7±0.61	6.07***
23	30.3±0.41	31.2±0.20	+0.9±0.46	1.96°
25	35.5±0.70	31.0±0.14	−4.5±0.71	6.34***
27	37.4±0.68	30.8±0.14	−6.6±0.69	9.57***
28	33.6±0.50	26.5±0.15	−7.1±0.52	13.65***
29	34.0±0.43	27.1±0.17	−6.9±0.46	15.00***
31	38.4±0.68	30.5±0.15	−7.9±0.70	11.29***
32	31.4±0.44	32.4±0.23	+1.0±0.50	2.00*
Mean	35.5±0.99	30.5±0.62	−5.0±1.12	4.46**

be cited the fact that the descendants of two A_1 plants with small cells do not show any decrease in cell size. In the opinion of the author, the present data definitely show that there is a decrease in cell size in the first few generations of descendants of A_1 plants with large cells.

The size of the pollen grains was determined mainly in Belling glycerine. This method is sufficiently exact for comparisons of pollen sizes between closely related materials that may be expected to react uniformly to the medium. It is not possible, however, to study the true shape of pollen in Belling glycerine. Whereas air dry pollen grains of *B. juncea* are decidedly ellipsoid, they become very nearly spherical in Belling glycerine.

The size of the pollen was measured on 20 A_1 plants and the mean diameter in Belling glycerine was 33.0±0.51 μ, whereas the corresponding value for natural *B. juncea* was 30.4±0.34 μ. The difference between the artificial and natural materials was fully significant ($P < 0.001$); FRANDSEN (1943), too, found that the pollen grains of artificial *juncea* are larger than those of the natural species.

In 1948 the pollen size was measured in four different varieties of natural *B. juncea* and in ten families of the artificial material, each family descending from one plant selected in 1947. Within each variety or family, 20 pollen grains were measured from each of 50 plants. The results of the measurements are summarized in Table 10, in which the families of artificial *juncea* were divided into two groups, B and C, the

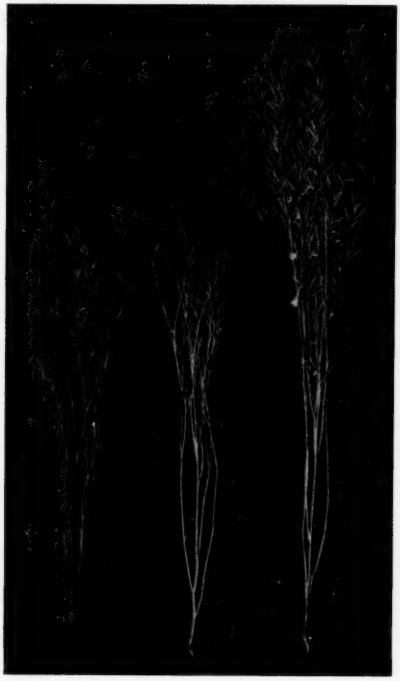


Fig. 11. A_3 plants of artificial *B. juncea*. The plant in the middle is strongly sterile, the other two plants are fertile.

former with a fertility above 90 per cent and the latter with a fertility below 90 per cent; it is of special interest to compare the pollen size of fully fertile artificial materials with that of the natural *juncea*. No difference exist between the groups A and B in Tables 10 and 11, whereas there is a marked tendency to a difference between these groups and the group C. Families with low pollen fertility have thus on an average somewhat larger pollen grains than those with full fertility. In the fertile families 91 and 96, the stomatal cells were measured as well as the pollen grains. The cells of family 91 were larger than those of family 96 both in regard to stomatal cells (Diff. $+1.33\,\mu$) and in regard to pollen ($+1.40\,\mu$). The less fertile family 75 had larger pollen grains than both 91 and 96 but was intermediate between these in regard to the size of the stomatal cells. Since there are statistically significant differences between families within groups in Table 11 ($P < 0.001$), it should be possible to

TABLE 10. *Pollen diameter in μ in natural and artificial B. juncea.* (Belling glycerine slides).

Material	n	% good pollen	Pollen diameter in μ $\bar{x} \pm m$
A. *Natural B. juncea*			
From Fiskeby	50	96.2	31.07±0.22
„ Hungary	50	97.0	29.83±0.21
„ Sophia	50	96.7	29.74±0.20
„ Brno	50	96.5	28.98±0.20
B. *Artificial B. juncea* > *90 % good pollen*			
A_4 family 1948 — 91	50	90.9	30.52±0.21
„ „ „ — 94	50	93.4	29.79±0.22
„ „ „ — 96	50	96.6	29.12±0.19
„ „ „ — 97	50	95.0	28.46±0.16
„ „ „ — 98	50	95.6	29.84±0.14
C. *Artificial B. juncea* < *90 % good pollen*			
A_4 family 1948 — 75	50	87.3	31.46±0.22
„ „ „ — 79	50	54.5	32.48±0.22
„ „ „ — 83	50	45.6	31.67±0.29
„ „ „ — 92	50	66.6	29.65±0.25
„ „ „ — 99	50	63.9	31.15±0.33

obtain varieties with different pollen sizes. The difference in pollen size between fully fertile and semisterile families of artificial *juncea* as well as the agreement in this respect between fertile artificial and natural material of *juncea* were confirmed by further measurements made in 1949.

In order to find out whether the agreement between artificial and natural *B. juncea*, found in the Belling glycerine slides, held true also with regard to the air dry pollen, measurements of the latter material were made in 1949. The study comprised 24 plants from each of a natural brown mustard from Sophia and a fertile A_8 family. One Belling glycerine preparation and one sample of air dry pollen were taken from the same flower. As seen in Table 12 the values obtained in the two types of preparations are in good agreement.

The measurements of stomatal length and of pollen diameter verified the preliminary results of FRANDSEN (1943) who found that the pollen of A_1 material of artificial *B. juncea* was larger than that of natural material. If the measurements are made in later generations of the artificial material, many families show, however, the same cell size as

TABLE 11. *Analysis of variance of the values for pollen diameter in Table 10.*

Cause of variation	DF	Sum of squares	Mean square	Quotient
Between groups	2	558.4372	279.2186	$\frac{279.2186}{54.8673}=5.09^{*}$
Within groups	697	2907.3350	4.1712	
Between A and B	1	19.0440	19.0440	
„ (A+B) and C	1	539.3932	539.3932	$\frac{539.3932}{54.8673}=9.83^{**}$
Between "varieties" within groups	11	603.5400	54.8673	$\frac{54.8673}{3.3583}=16.34^{***}$
Within varieties	686	2303.7950	3.3583	
Total	699	3465.7722		

varieties of the natural species. A few generations of cultivation obviously result in a decrease in cell size of the artificial material. The direct cause of this decrease was not ascertained, and no selection for smaller stomata cells or pollen grains was carried out. SCHWANITZ (1953) points out that the large cells are unfavourable to the plant and it is probable that biotypes with comparatively small cells, formed by recombination, would be indirectly favoured by a selection for high fertility.

4. Fertility and yield

A. *18- and 28-chromosome hybrid plants*

Eight out of the nine hybrid plants with 18 chromosomes had 0—1 per cent morphologically well-developed pollen, whereas one plant had

TABLE 12. *Comparison between pollen samples in Belling glycerine and dry samples from natural and artificial B. juncea.*

Type of sample	n	Pollen size in μ	
		Natural *B. juncea* $\bar{x} \pm m$	Artificial *B. juncea* $\bar{x} \pm m$
Belling glycerine, diameter	24	30.02±0.34	30.19±0.31
Dry samples, length	24	38.99±0.25	39.44±0.34
„ „ , breadth	24	21.11±0.12	21.08±0.16

10 per cent good pollen. The size of the pollen, as well as the variation in this quality, was considerably greater than in natural brown mustard. In the 28-chromosome plant there were only a few well-developed pollen grains (about 1 per cent) and many of these were large and had the appearance typical of dyad pollen.

After open pollination the 18-chromosome hybrids gave one single seed which developed into a 54-chromosome plant. After pollination with natural *juncea*, in an attempt to fertilize unreduced 18-chromosome egg cells, the F_1 plants gave 5 germinable seeds from 795 pollinated flowers. The plants obtained did not, however, have 36 chromosomes but around 54. At first metaphase these 54-chromosome plants showed not only bivalents and univalents but also fairly numerous trivalents. Evidently, they had three more or less complete sets of 18 chromosomes, the plants thus corresponding to triploid brown mustard. They probably originated from unreduced egg cells, the chromosomes of which had undergone one extra division (U, 1935). Morphologically, these plants were similar to normal brown mustard, but they were highly sterile and will not be dealt with further in this paper.

Pollination of the 18-chromosome hybrids with pollen from *nigra* did not result in any seeds. Pollination by *B. campestris* gave one F_1 plant which was morphologically identical with *B. juncea* and had 36 chromosomes. This plant may be a result of pseudogamy, shown to occur in *Brassica* by NOGUCHI (1928) and MOHAMMAD and SIKKA (1940), or of selfing. The 36-chromosome plant obtained is a typical artificial *B. juncea* and will be dealt with here under the number a.j. 24.

The few 54-chromosome seeds obtained from 18-chromosome hybrids were all derived from unreduced egg cells which had undergone one further doubling in chromosome number. That this hybrid, however, forms a majority of functional 18-chromosome gametes was demonstrated by RAMANUJAM and SRINIVASACHAR (1943). After open pollination of such plants, surrounded by *B. nigra*, *campestris* and *juncea*, these workers obtained offspring with 26, 28 and 36 chromosomes.

The 28 chromosome hybrid gave one single seed obtained after pollination with *B. campestris*. The plant had 21 (possibly 22) chromosomes and thus contained, besides two *campestris* genomes, one extra chromosome from *nigra*. It was fairly similar to *campestris* but was completely sterile and gave no seed.

B. *36 chromosome hybrids. Artificial B. juncea*

The A_1 material of a.j. 1—22, 23—24, 25—35 and 36—42 was

TABLE 13. *Pollen fertility of artificial and natural B. juncea. Variation between plants, families and generations.*

Material	Year and field no.	Frequency of plants with good pollen %																				Number of plants	$\bar{x}$ %
		5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100 %		
Artificial *B. juncea*, A_1	1945	2	2	1	2	4	1	5	1	1	1	1	1									22	26.6
,, ,, ,, A_2	1946		3	1	1	1	4	3	3	3	2	4	2	4	3	2	2	3	4			45	50.9
,, ,, ,, A_3 population	1947	10	7	1	7	3	4	8	7	4	10	10	5	7	6	7	10	8	6	3	3	126	49.6
,, ,, ,, A_3 family	1947—413—15	2	1	1	—	—	2	—	—	2	—	1	—	2	2	1	1	3	2			20	52.5
,, ,, ,, ,, ,,	—27						1	—	2	—	1	—	1	1	2	7	—	3	—	2		20	67.3
,, ,, ,, ,, ,,	—23							1	—	—	—	1	—	—	1	3	2	2	1	6	3	20	81.3
,, ,, ,, ,, ,,	—10														1	—	2	3	3	9	2	20	88.0
,, ,, ,, ,, ,,	—28											1	—	—	1	—	2	—	4	6	6	20	88.3
,, ,, ,, A_4 ,,	1948—83	4	1	4	4	2	3	3	4	3	3	1	1	1	2	2	2	3	3	—	4	50	45.8
,, ,, ,, ,, ,,	—92	1	1	2	2	1	1	2	2	1	1	2	2	2	2	1	3	3	6	6	9	50	66.8
,, ,, ,, ,, ,,	—93					1	—	—	1	—	—	1	1	—	3	1	4	3	8	7	20	50	86.0
,, ,, ,, ,, ,,	—94		1	—	—	—	—	—	—	—	—	1	—	—	—	—	—	2	1	8	37	50	93.2
,, ,, ,, ,, ,,	—96													1	—	—	1	1	—	3	44	50	95.8
Natural *B. juncea* from Hungary	1947—74																		1	8	11	20	95.0
,, ,, ,, ,, Sophia	1947—75																	1	4	10	5	20	92.3
,, ,, ,, ,, ,,	1948—88																			8	42	50	96.7
,, ,, ,, ,, Brno	1948—89																		1	8	41	50	96.5

obtained and studied in different years (*cf.* Table 3) and will be treated here separately. In order to obtain the largest possible quantities of seed from the highly sterile A_1 plants, artificial cross pollination was carried out to the largest possible extent within the different groups.

A.j. 1—22. In the A_1 material of 1945, the average pollen fertility of the 22 plants was only 26.6 per cent and the best plant had 56 per cent good pollen (Table 13). In A_2, the average pollen fertility was 50.9 per cent, and four plants had between 85 and 90 per cent good pollen. In 1947, the pollen fertility was studied in an A_3 population and in some A_3 families, descending from individual A_2 plants. The variation in pollen fertility within five of the A_3 families is given in Table 13. There is a considerable difference between different families, three out of 16 A_3 families having more than 80 per cent good pollen. In 1948 A_4 families obtained in the same way were studied; six out of 19 families had more than 90 per cent good pollen.

According to SCHULZ (1936) the number of ovules per pod is 20—24 in *B. campestris*, 12—20 in *B. juncea* and 7—11 in *B. nigra*. In the present investigation, the number of ovules (well developed + rudimentary seeds) was determined in 15—20 ripe pods from each of 50 plants of every species or family studied. The mean values obtained were 21.6 ovules per pod for *B. campestris*, 16.2 and 17.9 for two different varieties of *B. juncea*, respectively, and 10.4 for *B. nigra*. For three fairly fertile A_4 families, the values were 14.6, 15.9 and 16.8, respectively, and for two A_5 families, studied in the bud stage, 16.5 and 19.5, respectively. The number of ovules in artificial *juncea* falls within the limits given by SCHULZ for the natural species and is intermediate between the parents.

The female fertility was much more difficult to determine than the male fertility. In the former case, there was not only the haploid (gametic) sterility to consider, but also the diploid (zygotic) one, and probably here as in many other cases (MÜNTZING, 1933 b; WATKINS, 1932) disturbed balance between embryo and endosperm caused abortion of some seeds.

The seed fertility was determined by counting the number of well-developed seeds and more or less rudimentary seeds and ovules in the ripe pods. As already pointed out, it was difficult, however, to find all the rudimentary seeds and ovules in the ripe pods, and consequently the percentage of well-developed seeds might be a little too high (*cf.* p. 174). In order to obtain an estimate of how great a fraction of the original ovules were to be found in the ripe pods, the number of ovules per pod

TABLE 14. *Number of ovules per pod in the bud stage, ovules and seeds at ripening in artificial B. juncea.*

	n	A fertile family	A more sterile family	Difference	t
Number of ovules per pod in late bud stage	24	19.5±0.33	16.5±0.39	3.0±0.51	5.88***
Number of ovules+seeds per pod at harvest	24	17.9±0.45	11.5±0.58	6.4±0.73	8.77***
Number of well-developed seeds per pod at harvest	24	13.7±0.80	4.1±0.62	9.6±1.01	9.50***
Percentage well-developed seeds	24	74.9	35.6		
Number of well-developed seeds in per cent of ovules in bud stage	24	70.3	24.8		

were determined in two families of artificial *juncea*, first in the bud stage and later in the ripe pods. The results are summarized in Table 14. It can be seen that the seed fertility, estimated from analyses of the ripe pods, is too high. In the more fertile family the error is 4.6 per cent and in the more sterile one it is 10.8 per cent.

The differences between families and between generations are much greater, however, than the methodical error and in spite of their faultiness the estimates made give an acceptable picture of the possibilities of increasing the seed fertility. In Table 15, therefore, the seed fertility is reported for the A_1, A_2, A_3 and A_4 material whose pollen fertility is given in Table 13. Whereas there were on an average 6 per cent well-developed seeds in A_1, the corresponding value for A_2 was 26.5 per cent, and in the best A_3 and A_4 families 60—65 and 80—90 per cent, respectively.

The number of well-developed seeds per pod depends upon the number of ovules and upon the degree of fertility. There is a strong correlation between fertility and the number of well-developed seeds per pod (Table 17), and seed-setting is determined with much better reliability than fertility. The number of seeds per pod, therefore, offers a valuable complement to the estimate of fertility. Even the best A_4 families have a lower number of well-developed seeds per pod than good varieties of natural brown mustard, and this relation is further verified by counts in A_5 and A_6.

The effect of further selection in the following generations upon the number of seeds per pod was studied in 1955 by a comparison between A_4 material and A_7—A_9 material of some promising strains of artificial

TABLE 15. *Seed fertility in artificial and natural B. juncea. Variation between plants, families and generations.*

Material	Year and field no.	Frequency of plants with good seeds in %																				Number of plants	$\bar{x}$ %
		5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100 %		
Artificial *B. juncea*, A_1	1945	11 [1]	8	1	—	1	1															22	6.0
„ „ „ A_2	1946	8	3	3	3	4	—	4	7	3	5	3										43	26.5
„ „ „ A_3 population	1947	5	13	10	11	16	12	11	12	11	6	7	2	3	1	2	—	3				125	30.5
„ „ „ A_3 family	1947—413—15	3	4	2	3	2	6	6	4	4	3	2	3	3	1	1	1	1	1			50	36.7
„ „ „ „ „	—28					1	2	5	1	3	5	5	5	9	4	1	3	5	1			50	56.6
„ „ „ „ „	—21	1	—	—	2	2	—	1	—	2	2	7	5	7	5	5	8	2	1			50	59.2
„ „ „ „ „	—23			1	—	—	5	2	2	1	4	3	5	3	4	—	5	3	6	6		50	62.8
„ „ „ „ „	—10						1	1	1	1	2	3	9	6	12	4	4	2	4			50	64.2
„ „ „ A_4 „	1948—83	4	1	—	1	3	5	6	3	2	5	6	4	5	3	1	1					50	41.6
„ „ „ „ „	—92	2	—	—	—	1	1	3	—	1	2	3	2	1	3	5	9	8	7	2		50	66.9
„ „ „ „ „	—93								1	1	2	2	4	4	4	6	6	8	6	5	1	50	73.5
„ „ „ „ „	—94													1	2	7	8	9	8	12	3	50	83.4
„ „ „ „ „	—96														1	1	3	2	6	15	22	50	91.9
Natural *B. juncea* from Hungary	1947—74																1	5	4	28	12	50	92.0
„ „ „ „ Sophia	1948—88															2	—	—	1	10	37	50	95.3
„ „ „ „ Brno	1948—89																			11	39	50	96.4

[1] Of these, 7 with no well-developed seeds were counted as 0 when computing means.

TABLE 16. *Number of seeds per pod in 1955. Comparison between A_4 and A_7—A_9 material of artificial B. juncea and natural material.*

Material	A_4 material		A_7—A_9 material			Difference	t
	n	$\bar{x} \pm m$	Generation	n	$\bar{x} \pm m$		
Artificial *B. juncea*							
Sv 49/60	40	9.8±0.57	A_7	40	9.3±0.42	−0.5±0.71	0.70
Sv 50/62	40	14.1±0.30	A_7	40	13.2±0.21	−0.9±0.37	2.43*
Sv 50/63	40	9.8±0.44	A_8	40	12.9±0.31	+3.1±0.54	5.74***
Sv 50/64	40	8.3±0.43	A_8	40	11.9±0.43	+3.6±0.61	5.90***
Sv 54/247	40	9.1±0.51	A_9	40	14.2±0.38	+5.1±0.64	7.97***
Sv 54/248	40	6.1±0.38	A_9	40	10.8±0.41	+4.7±0.56	8.39***
Sv 54/249	40	9.7±0.46	A_9	40	13.3±0.48	+3.6±0.66	5.45***
Mean	7	9.6±0.91		7	12.2±0.64	+2.6±0.91	2.86*
Natural *B. juncea*							
From Stalingrad				40	13.2±0.36		
„ Hungary				40	10.3±0.37		
„ Brno				40	11.4±0.30		
„ Sophia				40	13.9±0.32		
Mean				4	12.2±0.82		

B. juncea. As seen in Table 16, five of the strains showed a very significant increase in number of seeds per pod in later generations, whereas the number decreased in two families. This decrease is probably only a random phenomenon, but it is possible that the A_4 material of Sv 50/62, which descended from a single highly fertile plant, had a better genetical constitution than later generations, where not only segregation but also crossing with related families have occurred. In spite of the differences between the strains, there is nevertheless a clear tendency to an increase in seed number in the later generations (Table 16) Furthermore, the A_7—A_9 material of artificial *B. juncea* had exactly the same number of seeds per pod as the mean of the four varieties of the natural species used for comparison.

In the A_3 and A_4 material studied here, pollen fertility, seed fertility and number of seeds per pod were determined both in the mother plant and in the progeny family, and it was possible to calculate the mother/progeny correlations for these characters. There was a highly significant correlation between the fertility of the mother and the progeny, apart from whether the measurement was based on pollen fertility, seed fertility or number of seeds per pod (Table 17). The strong correlation was

TABLE 17. *Correlations between different characters of fertility in artificial B. juncea.*

Character		n	b	r	t^2
Independent	Dependent				
Pollen fertility of the mother	Ditto of progeny	27	+ 0.60	+ 0.65	18.13***
Seed " " " "	" " "	29	+ 0.64	+ 0.81	51.51***
Number of good seeds of mother	" " "	20	+ 0.65	+ 0.85	46.86***
Seed weight per plant of mother	" " "	20	+ 0.13	+ 0.20	0.75
Number of good seeds per pod	Seed fertility, A_4 fam.	50	+ 5.64	+ 0.72	51.67***
" " " " " "	" " " "	50	+ 4.68	+ 0.81	91.58***
" " " " " "	" " " "	50	+ 3.06	+ 0.70	46.12***
" " " " " "	" " " "	50	+ 1.71	+ 0.67	39.10***
" " " " " "	" " " "	50	+ 1.53	+ 0.72	51.67***

partly due to the great variations in fertility of the material; in material with normal fertility there was also a correlation, but a weaker one between mother and progeny in regard to number of seeds per pod (OLSSON, 1960).

A.j. 25—32 and 34. Among the 9 A_1 plants in 1953, one (a.j. 28) was fully fertile with 98 per cent good pollen and 80 per cent good seed. In spite of the high fertility, this plant was beyond doubt an artificial product and not an admixture of natural *juncea*, since it deviated clearly from all available natural types by larger leaves, later development of the inflorescence and by rather short, broad pods. One of the remaining plants (a.j. 26) was lost before flowering. The mean pollen fertility of the remaining 7 plants was 16 per cent, and only very few ovules were developed into normal seed in spite of the fact that the plants flowered openly together with the fertile a.j. 28 and could have been pollinated by this plant.

Changes in the fertility of this material have been studied in A_2 and A_3, and the results correspond to those obtained in a.j. 1—22 (Table 18).

A.j. 36—42. The A_1 material was strongly sterile and only two of the seven A_1 plants gave any seed. The material was mostly used in an attempt to develop a winter form of *B. juncea*. The pollen fertility was determined in A_1, A_2 and A_3 and was, 23, 29 and 22 per cent, respectively; there was no average increase in fertility. Three plants in an A_3 family, however, had more than 80 per cent good pollen and probably it would be possible also in this material gradually to obtain an increase in fer-

TABLE 18. *Percentage of good pollen and number of seeds per pod in A_1 and A_2 and in the most fertile A_3 family of artificial juncea 25—34.*

Artificial *juncea* (a. j.) no.	Per cent good pollen					Number of good seeds per pod				
	A_1 plant	A_2 material		A_3 family		A_1 plant	A_2 material		A_3 family	
		n	$\bar{x}$	n	$\bar{x}$		n	$\bar{x}$	n	$\bar{x}$
25	28	7	59	34	72	0.1	9	2.3	51	6.3
27	32	6	57	36	63	0.1	11	4.5	55	6.7
28	98	20	95	20	92	16.6	24	11.7	40	14.7
29	2	20	57	13	85	0.1	23	3.0	40	7.5
30	3	—	—	—	—	—	—	—	—	—
31	2	2	11	15	40	0.1	8	2.8	40	7.6
32	2	—	—	—	—	0.1	1	9.1	16	4.7
34	44	—	—	—	—	0.1	—	—	—	—

Note: a.j. 30 gave no seeds in A_1.
a.j. 34 gave one A_2 plant which was destroyed by club root.

tility. Unfortunately, the most fertile plants were typical spring forms whereas the late plants (heterozygotes or pure winter forms) were highly sterile.

A.j. 23 and 24. The primary plant of a.j. 23 had 53 per cent good pollen and that of a.j. 24 had 43 per cent. The seed fertility amounted to only a few per cent. In the two following generations there occurred a marked increase in fertility, but the fertility then remained unchanged through A_3, A_4 and A_5 at levels indicated in Table 19.

As already mentioned, a.j. 23 is caused either by a somatic chromosome doubling at an early stage of the ontogeny or by the fusion of two unreduced gametes. In the former case the plant must be homozygous, in the latter it is heterozygous. A.j. 24 is the progeny of a diploid hybrid

TABLE 19. *Pollen fertility, seed fertility and number of seeds per pod in A_3—A_5 of a.j. 23 and 24, in comparison with natural B. juncea from Stalingrad.*

Material	Pollen fertility %		Seed fertility %		Number of good seeds per pod	
	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$
B. juncea from Stalingrad	200	91.9	150	84.4	200	15.6
Artificial *B. juncea*, a.j. 23	200	91.7	150	35.0	200	5.6
„ „ „ „ „ 24	200	85.6	150	49.6	200	8.4

and must be the result either of pseudogamy or of a fusion of two unreduced gametes in the mother plant; in both cases a.j. 24 must be homozygous. During the propagation, however, there has been a certain change of crossing between a.j. 23 and a.j. 24 and also between these and F_1 hybrids of natural *B. juncea* and a.j. 23 and 24. In any case, the possibilities for recombinations must have been much less in a.j. 23 and 24 than *e. g.*, within the artificial population a.j. 1—22, and this reduced possibility for recombination has evidently limited the effect of selection in a.j. 23 and 24.

Self-compatibility. B. campestris and *B. nigra* are as a rule self-incompatible. Natural brown mustard is generally self-compatible. In the strongly sterile A_1 material, it was impossible to determine the degree of self-compatibilty. The seeds obtained were nearly all the result of artificial cross pollinations and at least in most A_1 plants there was a clear tendency to prefer foreign pollen to that of the plant itself, whether this preference were caused by self-incompatibility or by any other circumstances. A.j. 23 and 24 gave, however, some seed also after selfing and the fertile A_1 plant a.j. 28 was fully self-compatible with 10.0 seeds per flower after isolation. After isolation in paper bags, some of the best strains of artificial *B. juncea* had as good a seed-setting as some of the natural varieties (Table 20).

Yield. Several varieties of natural *B. juncea* were tested in yield trials at Svalöf. Most of these varieties must be considered as land varieties whereas one variety, obtained from Professor Boguslawski, Giessen, was the result of systematic breeding work. Of the different local land varieties the brown mustard from Stalingrad was clearly superior to the others in seed yield as well as in yield of crude oil (ANDERSSON and OLSSON, 1953). This variety was, therefore, considered suitable as standard in judging the practical value of the artificial material. Table 21 gives the relative values for seed yield and yield of crude oil and also differences in content of crude oil, lodging resistance and weight of 1000 seeds for the best of the natural varieties and for some of the artificial ones, all in comparison with *B. juncea* from Stalingrad. Among the varieties of the natural species only the one bred by Boguslawski is somewhat superior to the Stalingrad strain in yield of seed and crude oil whereas several varieties have a higher percentage of crude oil in dry matter. The best artificial varieties are equal to the Stalingrad strain in yield of seed and crude oil, but they represent no improvement. Some of them are also equal in regard to oil content in dry matter, as for example Sv 50/63 in Table 21, but as a rule the

TABLE 20. *Seed-setting after bag isolation of natural and artificial B. juncea.*

Material	Field no. 1957	Number of isolated flowers	Number of seeds per isolated flower
Natural *B. juncea* from Stalingrad	1401— 1	127	11.8
„ „ „ „ Brno	16	101	11.8
„ „ „ „ Sophia	34	99	7.8
„ „ „ „ Giessen	68	147	6.5
Sv 51/189 from Fiskeby×Delft	71	178	10.6
Sv 49/60 from artificial *B. juncea*	1401—35	106	6.9
Sv 50/63 „ „ „ „	—36	126	7.6
Sv 54/248 „ „ „ „	—38	98	7.1
Sv 55/58 „ „ „ „	—39	127	8.4

artificial brown mustard has a lower oil content than the natural types. The selection so far mainly aimed at an increase in fertility and very little was done in regard to the oil content. Artificial as well as natural brown mustard was intermediate in oil content between the parent species. The differences between the species may be illustrated by the mean oil content from ten trials at Svalöf including one typical representative of each species.

Svalöf's Gute spring turnip rape	40.8 %
Stalingrad brown mustard	38.0 %
Weibull's black mustard	33.7 %

The lodging resistance was somewhat better in the artificial than in the natural material. The seed colour of the artificial *B. juncea* is brown just as in the parents and most natural varieties. According to FRANDSEN (1943), the seeds of artificial *B. juncea* are larger than those of the natural species. Also in the present material the seeds of the sterile families are fairly large. There is, however, a great variation in seed size and plants with extremely small seeds are also found. Table 22 gives the 1000 grain weight of three fairly fertile A_4 families of artificial *juncea* in comparison with two natural varieties. The seed size of the artificial material obviously falls within the range of the natural types. This is true also of the seed size of the material tested in variety trials (Table 21).

Also in some other characters, *B. juncea* is intermediate between *B. campestris* and *B. nigra*. *B. nigra*, *e.g.*, sheds the seeds considerably

TABLE 21. *Relative yields of the best natural varieties and the best artificial strains of B. juncea in relation to natural B. juncea from Stalingrad.*

Material	Number of trials	Relative value and difference from Stalingrad				
		Seed yield, rel. value	Oil content % in dry matter diff.	Oil yield, rel. value	Lodging resist.	1000 grain weight (g.) diff.
Natural B. juncea						
from Sophia	13	85	+ 2.1	90	+ 0.4	− 0.03
Boguslawski, Giessen	7	103	+ 0.5	104	± 0.0	+ 0.11
Fiskeby	6	84	+ 2.4	90	+ 1.5	− 0.56
Hungary	6	97	− 0.3	97	+ 0.2	+ 0.42
Rumania	6	97	− 0.5	97	− 0.3	+ 0.15
Brno	6	91	− 1.0	89	− 1.2	+ 0.17
Delft	6	83	+ 1.7	88	− 0.4	+ 0.10
Timisoara	5	84	+ 2.2	88	− 0.6	+ 0.06
Artificial B. juncea						
Sv 49/60	9	98	− 0.4	96	+ 0.3	+ 0.25
Sv 50/62	8	93	+ 0.3	94	+ 1.0	− 0.08
Sv 50/63	8	99	± 0.0	99	+ 0.4	+ 0.07
Sv 54/248	4	105	− 0.9	101	+ 0.8	+ 0.05
Sv 55/58=a.j. 28	3	77	− 3.8	69	+ 1.9	+ 0.07
Sv 53/235 from (artificial×natural from Brno)×natural from Hungary	5	99	+ 1.1	101	+ 0.1	+ 0.04

more than *B. campestris*; *B. juncea* is intermediate but more similar to *campestris* than to *nigra*. According to SYLVÉN (1949), *B. campestris* is heavily attacked by gall midge *Dasyneura brassicae Winn.*, while *B. nigra* is only rarely host for this insect. The varieties studied of *B. juncea* are obviously less suitable for the parasite than *B. campestris*, but they are more commonly attacked than *B. nigra*.

TABLE 22. *1000 grain weight in natural and artificial B. juncea 1948.*

Material	No. 1948	Number of plants	1000 grain weight (g.) $\bar{x} \pm m$
Natural *B. juncea* from Sophia	88	50	1.93±0.024
„ „ „ „ Brno	89	50	2.40±0.039
Artificial *B. juncea*, A_4 family	90	50	2.26±0.056
„ „ „ „ „	94	50	2.04±0.038
„ „ „ „ „	96	50	1.94±0.038

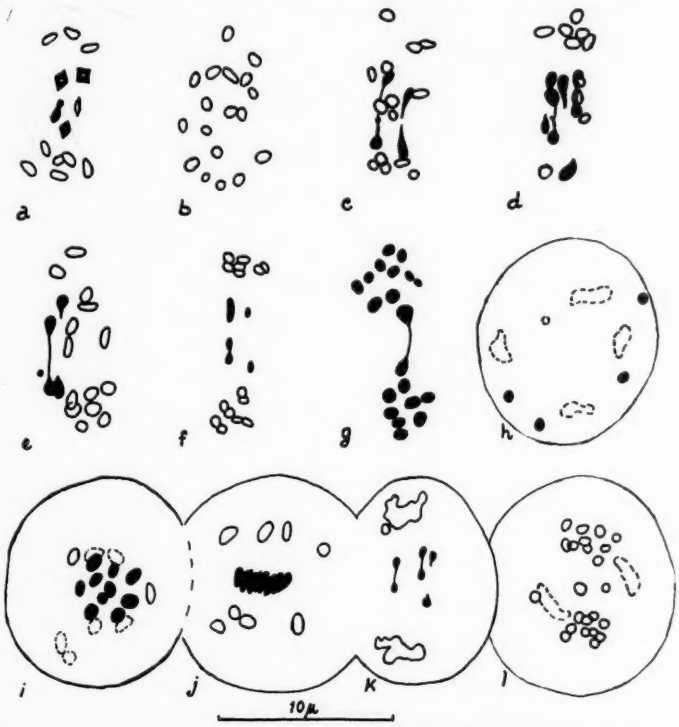


Fig. 12. a—h: meiosis of the 18 chromosome hybrid between *B. campestris* and *B. nigra*. a: diakinesis with 4_{II}; b: 18 univalents c—d: delayed separation of the bivalents; e: bridge with fragment; f: two divided and one undivided univalent in T_I; g: M_{II} with bridge remaining from A_I; h: T_{II} with several chromosomes irregularly distributed in the plasm.
i—l: meiosis of the 28 chromosome hybrid. i—j: M_I with $10_{II}+8_I$; k: T_I with three divided univalents; l: late A_{II} with vagabonding univalents, divided in first metaphase.

5. Cytology

A. *The 18-chromosome hybrids*

At diakinesis and first metaphase of the 18-chromosome hybrids studied here there are from $(0—5)_{II}$ and from $(18—8)_I$ (Table 23). In comparison it may be mentioned that U, MIZUSHIMA and SAITO (1937) obtained in the corresponding hybrid at most 3 bivalents, whereas RAMANUJAM and SRINIVASACHAR (1943) found in two cells as many as 7 bivalents. The latter authors also found isolated trivalents and even quadrivalents; in the present material there was possibly a trivalent in

a couple of cells. Stickiness and delayed terminalisation of the chiasmata made it difficult, however, to decide whether they were true trivalents. Both ring and rod bivalents occurred, the latter type being the most common one. Also obviously heteromorphous bivalents were observed (Fig. 12 a).

In some PMC's there are bridges with fragments at first anaphase (Fig. 12 e), and some of these bridges remain at second metaphase (Fig. 12 g). The delayed terminalisation of the chiasmata very often results in threads or bridges without fragments between the separating chromosomes at first anaphase (Fig. 12 c—d). The phenomenon has earlier been observed in haploid rape (OLSSON and HAGBERG, 1955) and is probably caused by non-homologous segments in the terminal or subterminal parts of the paired chromosomes. Some of the univalents are included undivided in the anaphase groups, whereas others divide at late anaphase and telophase. At second metaphase, the number of chromosomes vary between 1 and 18 with a mean of 9.8. The chromosomes occurring in the plates of second metaphase are not all equivalent, however, since they may derive from divided bivalents or from divided or undivided univalents. Second anaphase is, therefore, very irregular with remaining bodies — probably chromatids from univalents divided in first anaphase — between the separated anaphase groups. At second telophase there are also many chromosomes outside the telophase groups (Fig. 12 h). On the whole, the hybrid studied here shows the same meiotic irregularities as the corresponding hybrid studied by RAMANUJAM and SRINIVASACHAR (1943) and as the haploid rape studied by OLSSON and HAGBERG (1955).

The 18-chromosome hybrid between *B. campestris* and *B. nigra* corresponds in regard to its genomes to an amphihaploid *B. juncea*. A comparison in frequency of bivalents between the newly made hybrid and the amphihaploid studied by RAMANUJAM and SRINIVASACHAR (1943) and by MIZUSHIMA (1944) showed a higher frequency of bivalents in the hybrid than in the amphihaploid. A corresponding but weaker tendency to a higher frequency of bivalents in the new hybrid than in the amphihaploid was also found in the 19-chromosome hybrid between *B. campestris* and *B. oleracea* as compared with amphihaploid *B. napus* (MORINAGA and FUKUSHIMA, 1933; U, 1935); and the same was true of the 17-chromosome hybrid between *B. oleracea* and *B. nigra* and amphihaploid *B. carinata* (MIZUSHIMA, 1950; KURIYANA and WATANABE, 1955). OLSSON and HAGBERG (1955) in their amphihaploid *B. napus* obtained approximately the same frequency of bivalents as reported by

TABLE 23. *Frequency of different configuration at first metaphase in the 18-chromosome hybrid between B. campestris and B. nigra.*

Configuration	Number of cells
$5_{II}+8_{I}$	5
$4_{II}+10_{I}$	8
$3_{II}+12_{I}$	12
$2_{II}+14_{I}$	5
$1_{II}+16_{I}$	5
$0_{II}+18_{I}$	10
Total	50

U (1935) from his hybrid, but on the other hand the former authors (unpubl. data) obtained a still higher bivalent frequency in a new hybrid. In spite of the uncertainty of comparisons between hybrids and amphihaploids studied at different times in widely different places, it seems to be possible to trace in the amphidiploid a tendency to a differentiation of the subgenomes towards a lower degree of homology.

The bivalents of the 18-chromosome hybrid may be the result either of auto- or allosyndesis. The possibility of autosyndesis is indicated by the occurrence of secondary pairing in *campestris* (CATCHESIDE, 1934; ALAM, 1936) and in *nigra* (HAGA, 1938; SIKKA, 1940). On the other hand, RAMANUJAM (1941) found in haploid *campestris* a single bivalent only in a few cells. So far as is known to the author, haploid *nigra* has not been studied, but in hybrids with a single *nigra* genome the author has not found any case of autosyndesis between the *nigra* chromosomes (see further below). According to MIZUSHIMA (1950), the possibility of one autosyndetic pair in the *nigra* genome and of two in the *campestris* genome cannot be theoretically excluded but is considered to be very rare in nature. The formation of bivalents in the hybrid is much more common than expected from only autosyndetic pairing. Consequently, we must conclude that allosyndesis also occurs, and that the great majority of the bivalents are the result of pairing between one chromosome from each of the *campestris* and *nigra* genomes.

B. *The 28-chromosome hybrid*

The meiosis of the 28-chromosome plant is particularly characterized by the general occurrence of $10_{II}+8_{I}$ at first metaphase (Fig. 12 i—j). As already mentioned, the morphology of the plant indicated that it should contain 10+10 *campestris* chromosomes and 8 *nigra* chromo-

TABLE 24. *Number of bodies at first metaphase in different generations of artificial B. juncea and in natural material.*

Material	Number of bodies per cell at M_I							Number of cells studied
	17	18	19	20	21	22	23	
Artificial *juncea*, A_1	2	140	66	38	18	9	2	275
„ „ , A_4+A_5	3	213	28	6				250
Natural *juncea*		96	3	1				100

somes, and this assumption was verified by an analysis of the meiosis. On the other hand, no pairing has been observed among the 8 *nigra* chromosomes, and in any case there is no strong tendency to autosyndesis between them. The disturbances in the meiosis of the 28-chromosome plant fully correspond to those described by MORINAGA (1929 b) in the hybrid between *juncea* and *campestris*, a matter which will be further discussed on page 216.

C. *36-chromosome amphidiploid*

With the method used, it has not been possible to analyse in detail the pairing at diakinesis and first methaphase. The study of these stages must be confined to a determination of the total number of bodies in the metaphase plate visible in polar view (number of univalents+bivalents+multivalents) and the number of bodies outside the metaphase plate seen in side view. In general, the frequency of multivalents is very low, only a small number of probable trivalents having been observed. In cases where 19 or 20 bodies were counted in polar view of first metaphase this was probably nearly always corresponding to $17_{II}+2_I$ and $16_{II}+4_I$, respectively. In a few cells only 17 bodies were seen, but this was not necessarily due to multivalent formation since there is also the possibility that some of the small bivalents escaped observation. The number of bodies counted in M_I is given in Table 24. Usually there

TABLE 25. *Number of chromosomes at second metaphase of different generations of artificial B. juncea and in natural material.*

Material	Number of chromosomes per cell in M_{II}											Number of cells studied
	14	15	16	17	18	19	20	21	22	33	36	
Artificial *juncea*, A_1	1	6	15	49	124	35	13	4	1	1	1	250
„ „ , A_4+A_5			3	25	210	12						250
Natural *juncea*				6	91	3						100

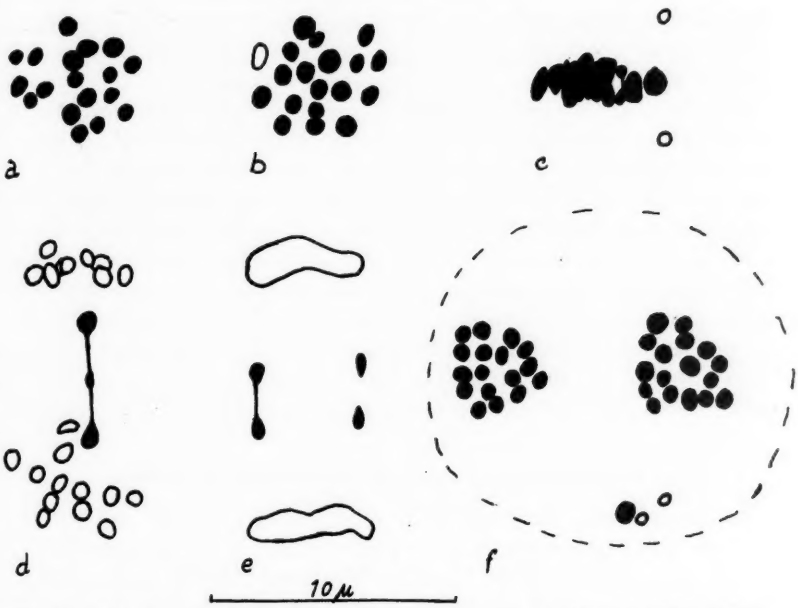


Fig. 13. Meiosis of artificial *B. juncea*. a: 18_{II}; b—c: $17_{II}+2_I$; d: bridge without fragment; e: two univalents divided in T_I; f: M_{II} with 17 chromosomes in the plates and one divided and one undivided univalent outside the plates.

are 18 bivalents, but there is also a fairly high frequency of univalents, especially in A_1. In A_4+A_5 the meiosis is much more regular than in A_1 but still more disturbed than in natural *juncea*. If the material is divided into two groups with 17—18 bodies and with more than 18, respectively, the difference between, on the one hand, A_4+A_5 and, on the other, natural *juncea* will give a χ^2 of 6.80**. A corresponding comparison between A_1, on the one hand, and A_4+A_5, on the other, gave a χ^2 of 69.4***.

At first anaphase of the A_1 generation, single bivalents showed in some cells bridges without fragments of the same type as observed in the 18-chromosome hybrid (Fig. 13 d). Thus, it is probable that at least in A_1 plants there is a low frequency of pairing between *campestris* and *nigra* chromosomes. In many cells, at late anaphase and telophase of the A_1 material, divided univalents are left between the anaphase groups (Fig. 13 e).

The number of chromosomes at second metaphase are given in Table 25. If the material is divided into two classes with 18, and with

more or fewer than 18 chromosomes, respectively, the comparison between A_1 and A_4+A_5 gives a χ^2 of 65.9***. A corresponding comparison between A_4+A_5 and natural *juncea* gives a χ^2 of 2.08.

Summarizing the above results, it may be stated that artificial *B. juncea* shows considerable meiotic disturbances in the first generation but a marked stabilization already after 4 to 5 generations. The regularity of the natural species is, however, not reached. There are considerable differences between different A_1 plants, however, and the fully fertile a.j. 28 has a nearly completely normal meiosis. FRANDSEN (1943) as well as RAMANUJAM and SRINIVASACHAR (1943) report fewer meiotic disturbancies in their material than were found on an average in the present material. On the whole, the meiotic disturbances are less than would be expected from the high degree of sterility in A_1, and a considerable part of the reduction in fertility must be due to other causes.

6. Crosses between artificial and natural B. juncea

In order to test the possibilities for crossing artificial and natural *B. juncea*, and also with a view to further breeding work, a number of crosses were made between the artificial material and natural varieties. The artificial material was consistently used as a mother in order to avoid any admixture of natural material due to unsuccessful emasculation. In Table 26 data are given for the seed-setting of the artificial material after open flowering in a stand of such material and after artificial pollination with natural material. As seen from the table, the seed-setting is considerably improved by pollination with natural *juncea*

TABLE 26. *Seed-setting in A_1 material of artificial B. juncea after pollination with natural B. juncea.*

Cross combination	Year	Crosses			Number of seeds per flower after free flowering in A_1
		Number of flowers pollinated	Number of good seeds	Number of seeds per flower	
A.j. 1, 2, 5 and 12×*B. juncea* from Brno	1945	41	52	1.3	0.24
A.j. 23×*B. juncea* from Stalingrad	1949	8	49	6.1	0.09
A.j. 23×A_5 material of a.j. 11	„	20	89	4.5	0.09
A.j. 25, 27, 29—32, 34×*B. juncea* from Stalingrad	1953	95	124	1.3	0.10
A.j. 28×*B. juncea* from Stalingrad	„	10	145	14.5	16.6

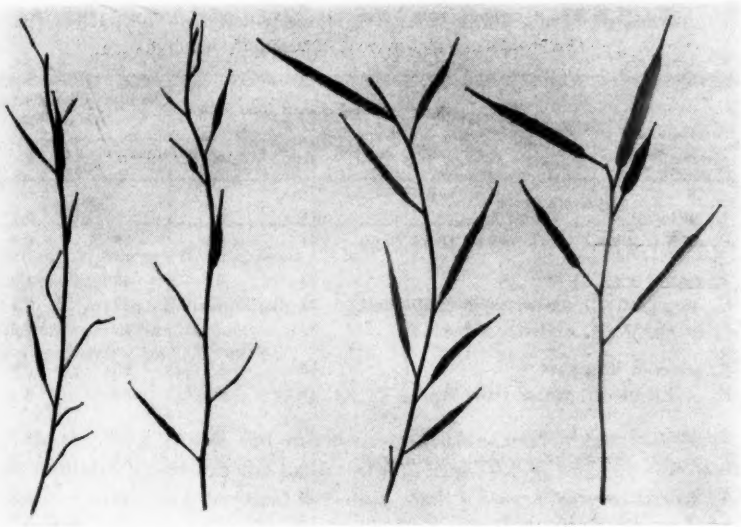


Fig. 14. Pod development in A_1 of a.j. 23, after selfing (left), after pollination with natural *B. juncea* (right).

(Fig. 14). Pollen from a fairly fertile A_6 family had the same favourable effect upon the seed-setting. Also when A_2 and A_3 were pollinated with natural *juncea*, the seed-setting was better than after open flowering in an artificial population, although the difference here was less than in the more sterile A_1.

The introduction of a genome from natural *B. juncea* in the highly sterile A_1 results in an improved balance, which is shown not only by the decrease in zygotic lethality and improved seed-setting but also in a higher fertility of the F_1 material as compared with the corresponding A_2 material. Table 27 gives the pollen fertility and the number of well-developed seeds per pod for a number of F_1's of the cross $A_1 \times$natural *juncea* and for the corresponding A_2 generations. Except in the case of the fully fertile a.j. 28, F_1 has a higher pollen fertility and a greater number of good seeds per pod than A_2.

In F_2 and F_3, there was a wide variation in colour, shape, and size of leaf, height of plant, time of flowering and ripening, lodging resistance, pod size, fertility *etc.* The material from the crosses between artificial and natural *B. juncea* was mainly used for practical breeding work and the fertility was analyzed only in five F_3 families of the cross arti-

TABLE 27. *Comparison between A_2 material and F_1 from the cross artificial A_1×natural B. juncea.*

Cross combination	Per cent good pollen		Number of good seeds per pod	
	n	$\bar{x}$	n	$\bar{x}$
A_2 material of a.j. 2 and 5	8	54	6	0.7
F_1 of a.j. 2 and 5×*B. juncea* from Brno	44	66	13	4.7
A_2 material of a.j. 23	47	87	45	1.9
F_1 of a.j. 23×*B. juncea* from Stalingrad	32	95	27	9.7
F_1 of a.j. 23×A_2 material of a.j. 11	55	95	47	7.9
A_2 material of a.j. 24	15	85	15	3.9
F_1 of a.j. 24×*B. juncea* from Brno	19	93	18	8.1
A_2 material of a.j. 28	20	95	24	11.7
F_1 of a.j. 28×*B. juncea* from Stalingrad	17	90	17	11.2
A_2 material of a.j. 29	20	57	23	3.0
F_1 of a.j. 29×*B. juncea* from Stalingrad	11	59	11	4.2

ficial *juncea*×*juncea* from Brno (Table 28). Seed fertility and number of good seeds per pod proved to be poorer than in brown mustard from Stalingrad and resembled more the fertiliy of the A_4 families in Table 15. A number of strains from crosses between artificial material and several natural varieties were tested in variety trials, but only in very rare cases were they equal to the best artificial strains. The introduction of a natural *juncea* genome into the sterile A_1 material greatly improved the fertility,

TABLE 28. *Pollen fertility, seed fertility and number of seeds per pod in F_3 families of the cross artificial×natural B. juncea and in natural B. juncea.*

Material	Field no. 1948	Number of plants	Good pollen %	Good seed %	Number of well-developed seeds per pod
Artif.×natural *B. juncea*, F_3 fam.	423— 5	50	87.4	76.0	12.0
„ × „ „ „ „ „ „	423—10	50	71.4	61.5	9.2
„ × „ „ „ „ „ „	423—19	50	96.9	78.8	11.3
„ × „ „ „ „ „ „	423—23	50	91.3	73.7	9.4
„ × „ „ „ „ „ „	423—28	50	91.9	71.6	11.1
Natural *B. juncea* from Stalingrad	423—11	50	96.2	94.2	17.0

TABLE 29. *Crosses between artificial and natural B. juncea, respectively, and B. campestris.*

Cross combination	Number of flowers pollinated	Number of F_1 plants per 100 pollinated flowers	Number of plants with chromosome number determined	Chromosome number of the F_1 plants
Natural *B. juncea*×*B. campestris*	428	53.7	64	63 pl. 2n=28 1 pl. 2n=38
Artif. *B. juncea*×*B. campestris*	424	17.7	50	2n=28
B. campestris×Nat. *B. juncea*	550	6.2	31	2n=38
B. campestris×Artif. *B. juncea*	413	2.4	7	6 pl. 2n=38 1 pl. 2n=28

but in the long run it did not prove advantageous in comparison to selection strictly within the artificial material. When judging the possibilities of obtaining practical breeding results by crossing artificial and natural *B. juncea*, it should be observed, however, that the crosses here discussed were made with artificial material in A_1, A_2 and A_3; crosses with fully fertile material might have yielded better results. Further, it might be possible, by repeated backcrossing, to introduce specially desirable characters from the artificial material into good natural varieties.

V. CROSSES BETWEEN B. JUNCEA AND B. CAMPESTRIS

The results of the crosses between *B. juncea* and *B. campestris* are given in Table 29. These crosses produce considerably less seed than *e. g.*, crosses between *B. napus* and *B. campestris* (SINSKAIA, 1927; OLSSON, 1949), but considerably more than the already discussed crosses between diploid *campestris* and *nigra*. As seen from the table, the seed-setting is considerably better when *juncea* is used as mother than in the reciprocal combination. There is also less seed-setting when artificial *juncea* is crossed with *campestris* than when natural *juncea* is used. The lower fertility of artificial *juncea* may in itself contribute to this difference, but it can be safely stated that the sterility barrier between artificial *juncea* and *campestris* is as evident as when the *juncea* component is a natural type.

The chromosome number in F_1 was not always the same; plants occurred with 28 (18+10) as well as with 38 (18+20) chromosomes in

TABLE 30. *The size of matromorphous seeds and hybrid seeds from natural and artificial B. juncea crossed with B. campestris.*

Cross combination	Chromosome number	Number of seeds	Weight of one seed in mg $\bar{x} \pm m$
Natural *B. juncea*×*B. campestris*			
Matromorphous seeds	36	68	2.74±0.11
Hybrid seeds	28	63	0.80±0.031
„ „	38	1	2.6
Artificial *B. juncea*×*B. campestris*			
Matromorphous seeds	36	15	2.47±0.19
Hybrid seeds	28	50	0.68±0.042
B. campestris×natural *B. juncea*			
Matromorphous seeds	20	43	2.30±0.11
Hybrid seeds	38	29	1.99±0.091
B. campestris×artificial *B. juncea*			
Matromorphous seeds	20	21	1.72±0.19
Hybrid seeds	28	1	1.7
„ „	38	6	1.78±0.27

root-tips. In cases where *B. juncea* was used as the mother, the chromosome number was determined in 114 plants, 113 of which had 28 chromosomes whereas one had 38. In the reciprocal combination, with *B. campestris* as mother, only one plant had 28 chromosomes, all the other ones having 38 (Table 29). Evidently, when *campestris* is used as mother, nearly always unreduced female gametes function and both gametes have about the same chromosome number. The fact that one 38-chromosome plant was obtained, when *campestris* was used as father, showed, however, that some of the male gametes of this species were also unreduced. That plants with 38 chromosomes are rather rare in this combination is most easily explained by the assumption that haploid pollen tubes grow more rapidly than diploid ones and that the former type will, therefore, prevail in fertilizing the eggs (*cf.* JULÉN, 1950).

According to SIKKA (1940), the F_1 seeds obtained from the cross *B. juncea*×*campestris* were much smaller than the seeds of the parent species. An account of this and also of the results of HOWARD (1939, 1942) and the present author (OLSSON and RUFELT, 1948) the seed size was studied. The results are given in Table 30. The 28-chromosome F_1 seeds of the cross *B. juncea*×*campestris* are evidently considerably smaller than the matromorphous seeds from the same plants (Fig. 15).

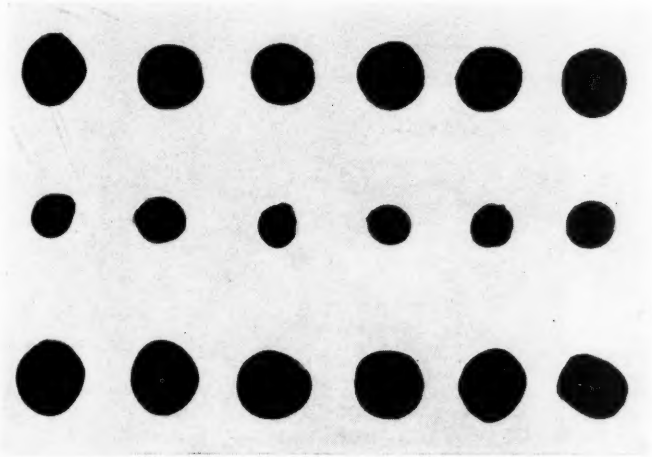


Fig. 15. Seeds of *B. juncea* from Brno (above), *B. campestris* (Mette's, below) and F_1 seed from the hybrid between them (middle) (enlarged about × 6).

The 38-chromosome seeds from the reciprocal cross are, on the other hand, of about the same size as the matromorphous seeds.

Morphologically, the F_1 plants are intermediate between the parents (Figs. 16—17) with the *campestris* characters much more prominent in the 38 chromosome plants than in those with 28 chromosomes.

The pollen fertility was poor in all the material, but varied strongly between different plants. In the cross natural *juncea*×*campestris*, 174 examined plants had on an average 19 per cent good pollen. The corresponding mean for the combination artificial *juncea*×*campestris* (49 plants) was 13 per cent. In 32 plants of the reciprocal combination *campestris*×natural *juncea* the mean pollen fertility was 26 per cent, with variations between the plants from 10 to 45 per cent. In 10 plants of the corresponding cross with artificial *juncea*, the mean fertility was 20 per cent.

The seed fertility was very low; at open flowering most plants gave no seed what so ever, and some plants yielded a very few seeds. The frequency of plants with seed was somewhat higher in the cross *campestris*×natural *juncea* than in the other combinations. On the whole, the production of well-developed seeds was considerably below 1 per cent.

The meiosis of the 28-chromosome hybrid between natural *B. juncea* and *B. campestris* was treated in detail by MORINAGA (1929 b), SIKKA (1940) and RAMANUJAM and SRINIVASACHAR (1943). The author's own

Fig. 16. *B. juncea* (left), *B. campestris* (right), the 28 chromosome hybrid between them (middle).

observations agree with the results of the authors mentioned. The meiosis is characterized by $10_{II}+8_{I}$ at first metaphase and corresponds to the *Pilosella*-type of TÄCKHOLM (1922). Already MORINAGA (1929 b) concluded that 10 of the *juncea* chromosomes were homologous to the *campestris* chromosomes. The 28-chromosome F_1 plants from the cross artificial *juncea*×*campestris* showed the same type of meiosis as the hybrids between natural *juncea* and *campestris*. The 38-chromosome plants from the reciprocal cross were mainly characterized by a relatively high frequency of trivalents.

Briefly, it may be said that the crosses between artificial and natural *B. juncea*, on the one hand, and *B. campestris*, on the other, have shown:

1. that when *B. juncea* is used as mother almost only 28-chromosome hybrids are formed, whereas 38-chromosome F_1's are equally prevalent in the reciprocal combination;

2. that F_1 seed giving rise to 28-chromosome plants are considerably smaller than the seeds of the mother species;

3. that the material of *B. juncea* studied here contains one *campestris*

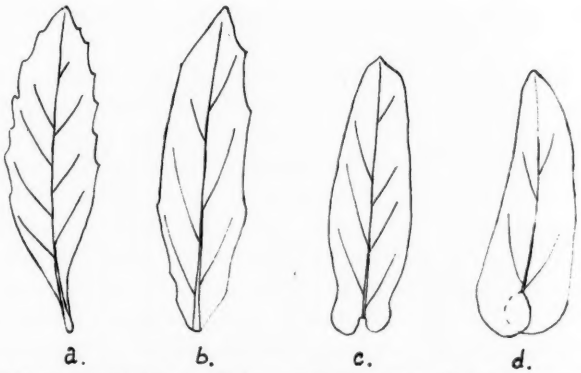


Fig. 17. Upper stem leaves. a: *B. juncea*; d: *B. campestris*; b: 28 chromosome hybrid; c: 38 chromosome hybrid between them.

genome, just as was the case with the material studied by MORINAGA (1929 b) and other authors;

4. that artificial *juncea* behaves in the same manner as natural *juncea*, in crosses with *campestris*.

VI. CROSSES BETWEEN B. JUNCEA AND B. NIGRA

According to SINSKAIA (1927), *B. juncea* and *B. nigra* cannot be crossed. From 201 *juncea* flowers, pollinated with *nigra*, however, MORINAGA (1934) obtained one F_1 plant. The cross was later investigated by RAMANUJAM and SRINIVASACHAR (1943).

From 613 flowers of natural *B. juncea* pollinated with *nigra*, the present author obtained 6 F_1 plants, 4 of which had 26 (18+8) chromosomes, whereas two had 34 (18+16). Neither the reciprocal combination nor crosses with artificial *juncea* yielded any seeds (Table 31). Thus

TABLE 31. *Crosses between natural and artificial B. juncea, respectively, and B. nigra.*

Cross combination	Number of pollinated flowers	Number of seeds harvested	Number of F_1 plants
Natural *B. juncea*×*B. nigra*	613	203	6
Artificial *B. juncea*×*B. nigra*	497	21	0
B. nigra×natural *B. juncea*	618	46	0
„ „ ×artif. „ „	295	7	0

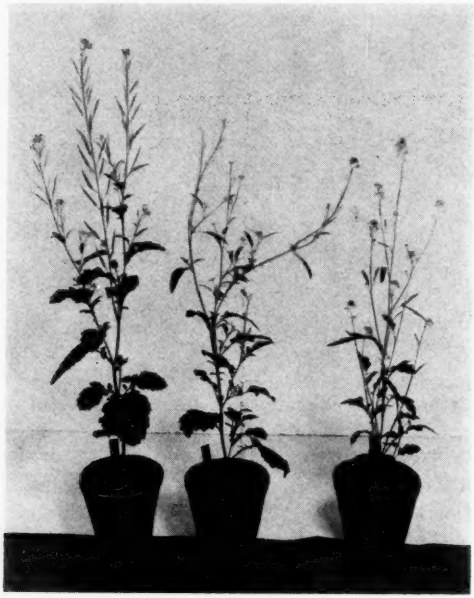


Fig. 18. *B. juncea* (left), *B. nigra* (right), the hybrid between them (middle).

Fig. 19. Upper stem leaves. a: *B. juncea*; c: *B. nigra*; b: 26 chromosome hybrid between them.

artificial *juncea* is as markedly separate from *nigra* as is the natural species.

The F_1 plants are intermediate between the parents (Figs. 18—19); the 34-chromosome plants tending somewhat more towards black mustard than the 26-chromosome ones. The plants are highly sterile with only 5—10 per cent well-developed pollen and none or very few well-developed seeds.

The meiosis of the 26-chromosome plants is characterized by $8_{II}+10_{I}$ at first metaphase, and in the following stages the univalents behave just as those in the 28-chromosome *juncea*×*campestris* hybrid. These observations agree completely with those of MORINAGA (1934), 8 of the *juncea* chromosomes undoubtedly being homologous to the *nigra* chromosomes. The 34-chromosomes plants are characterized zy an increase in the frequency of trivalents.

VII. SUMMARY

The present paper deals with different characters of artificial *B. juncea*, similarities and differences between natural and artificial *juncea* and the utilization of the artificial material in practical breeding work. It is the author's intention in another paper to deal with the corresponding questions in artificial *B. napus*, and it has been considered suitable to postpone the general discussion of the results until both materials have been reported, especially as the results with *juncea* have already been discussed to some extent in the previous sections of this paper.

The results may be briefly summarized in the following points:

1) *B. campestris* and *B. nigra* can be crossed on the diploid as well as on the tetraploid level. Crosses on the tetraploid level result in artificial *B. juncea*. On one occasion artificial *juncea* appeared directly from a cross between diploids.

2) Artificial *B. juncea* is intermediate between the parent species and agrees very well with the natural species.

3) The cell size of the newly synthesized material is considerably larger than in the natural species, but this difference is for the most part eliminated already after cultivation for a few generations.

4) Fertility in the new artificial material is in most cases very low. Four to five years of continuous selection increases fertility almost to the level of natural material.

5) The seed yield of the best strains of artificial *B. juncea* is about

the same as that of good, non-bred, local varieties, whereas the oil content is as a rule somewhat lower.

6) The 18-chromosome hybrids have a very irregular meiosis, with $(0—5)_{II}+(18—8)_{I}$.

7) In the first generation, the artificial *B. juncea* examined generally showed pronounced meiotic disturbances, but already in the fourth and fifth generation meiosis was markedly stabilized, though not quite attaining the perfect regularity of the natural material.

8) Crosses between artificial and natural *B. juncea* succeed without difficulty and the hybrids are fully fertile.

9) The sterility barriers between artificial *juncea* and the parent species, *campestris* and *nigra*, are at least as strongly marked as in the case of natural *juncea*.

10) In the cross *juncea*×*campestris* hybrids seeds with 28 chromosomes are obtained, which are smaller than the nonhybrid seeds of the female parent. In the reciprocal cross F_1 seeds of normal size are produced, almost all of them having 38 chromosomes.

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SOMATIC SEGREGATION IN TOMATO

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THE occurrence of somatic reduction of chromosomes followed by restoration to the diploid number in colchicine-induced tumors within sorghum coleoptiles has been postulated by FRANZKE and ROSS (1952) and ROSS *et al.* (1954) to account for the true-breeding nature of mutant shoots growing out of these tumors. Since sorghum may be of polyploid origin, viable mutants might result from abnormal distribution of chromatin during somatic reduction. This hypothesis was later modified to include mutagenesis when HARPSTEAD *et al.* (1954) found that no detectable relocation or duplication of chromatin had taken place in the mutants. If the somatic reduction phenomenon is of general occurrence, then evidence in the form of homozygous shoots or sectors might be expected in the subsequent growths after treatment of heterozygous growing points in other species. Easy identification of heterozygous and homozygous tissue containing the aurea mutant in tomato, *Lycopersicon esculentum*, suggested the possible usefulness of this material for testing the hypothesis of somatic reduction.

MATERIALS AND METHODS

The aurea mutant of tomato was discovered and described by Dr. A. PERSSON (1959), who selected it after irradiation of seed. After X-ray treatment of soaked seeds with 10,000 r, a variegated X_1 plant appeared which had aurea as a ground color with green and lighter patches occurring in it. Progeny of this plant segregated in the ratio of 1 green (++): 2 *aurea* (A+): 1 yellow (AA). The yellow seedlings developed no chlorophyll and later died. The heterozygote was as vigorous as the normal, but could be recognized immediately by its pale green color. Of the *aurea* plants 0.2 % were observed to have single green spots of variable size on one or more leaves. It was of interest

[1] This work was carried out while a Guggenheim Fellow at the Institute of Genetics, University of Lund, Sweden.

Fig. 1. Leaves from colchicine-treated tomato plants heterozygous (+*A*) for the *aurea* mutant showing a green spot on the leaf at the left and twin green and yellow spots on the leaf at the right. Similar spots were also found on untreated plants. These spots appear to have the same phenotype as the green (++) and yellow (*AA*) seedlings obtained in the F_2 progeny.

that this phenomenon was more noticeable in tetraploid plants where twin spots of green and yellow, as well as single spots of either type were noted.

Colchicine (0.5 %) in lanolin was applied to the epicotyls of very young seedlings. After treatment they were placed under an infrared lamp at a temperature of 26° C for 7 days and then grown to maturity

TABLE 1. *Comparison of the number and size of spots including green, yellow and twin spots between colchicine-treated and untreated tomato plants heterozygous for the aurea mutant.*

	Number of plants	Spots per leaf		Spot size	
		Mean	Range	Mean	Range
Treated	33	0.10	0.03—0.18	28.51	0.09—618.99
Untreated	33	0.06	0.02—0.11	1.96	0.06— 19.25
Difference		0.04**		26.55**	

** $P<0.01$ (t test).

in a greenhouse. True-breeding mutants had previously been produced in sorghum following a similar exposure to infrared after colchicine application (unpublished). Thirty-three plants were treated while the same number of untreated plants were kept as controls. The untreated plants were not subjected to infrared irradiation and were necessarily treated somewhat differently from the standpoint of culture.

Examination for evidence of somatic segregation was made when the plants had approximately 200 leaves. The total number of spots, both single and twin, were counted and the number of leaves per plant noted. Approximate measurements on each spot were made in millimeters.

RESULTS AND DISCUSSION

The nature of the spots on leaves of plants heterozygous for the aurea mutant is illustrated in Fig. 1. Both the leaves were taken from colchicine-treated plants, but similar spots were found on untreated plants as well. The contiguous nature of the twin spots indicates the apparent origin of both from the same somatic segregation.

As shown in Table 1, counts of the number of spots per leaf on each plant varied from 0.03—0.18 with a mean of 0.10 for the treated plants while the untreated varied from 0.02—0.11 with a mean of 0.06. The highly significant difference ($P<0.01$) between these means indicates that the treatment caused an accentuation of the phenomenon by which these spots of homozygous tissue are formed. The difference in size of spot is also highly significant indicating segregation must take place earlier in the development of the treated plant than in the untreated plant. The ratio of yellow to green spots (Table 2) though higher in the treated plants, does not differ significantly from the untreated as shown by the χ^2 test.

TABLE 2. *Comparison of the ratio of green to yellow spots between colchicine-treated and untreated tomato plants.*

Color of spots	Treated	Untreated
Green	442	337
Yellow	31	13

$\chi^2=2.37$; P=0.50.

It is possible that the low ratio of yellow to green is due to the inability of the examiner to differentiate between the yellow spots caused by injury and those resulting from segregation of the *aurea* gene, but it is also possible that the homozygous *aurea*, because of its lethal nature, did not have the same competitive advantage as the homozygous green tissue. This would partially account for the low ratio of double to single spots as shown in Table 3. No significant difference was found between the ratio in the treated and control plants.

It was found that the per cent good pollen differed significantly between the treated plants and the controls (Table 4). This difference could be caused by pollen sterility resulting from polyploidy in the treated plants. Cytological examination of two treated plants, one of which had 70.89 % and the other 81.44 % good pollen, showed that both were tetraploid. However, when correlation coefficients between pollen fertility and the number of spots per leaf and size of spots were computed, they were found to be low and not significant as shown in Table 5. If low pollen fertility is equated with tetraploidy, tetraploidy does not appear to have increased the number or size of spots. No final conclusion, however, can be drawn.

The presence of homozygous single spots of tissue for green (++) and yellow (AA), as well as the contiguous double spots, demonstrates

TABLE 3. *Comparison of the ratio of double to single spots between colchicine-treated and untreated tomato plants.*

	Number of plants	Ratio of double spots/single spots	
		Mean	Range
Treated	33	0.30	0.00—0.67
Untreated	33	0.22	0.00—1.00
Difference		0.08	

No significant difference (t test).

TABLE 4. *Comparison of per cent good pollen between colchicine-treated and untreated tomato plants.*

	Number of plant	Per cent good pollen	
		Mean	Range
Treated	22	91.44	98.60—59.26
Untreated	29	97.12	98.73—76.45
Difference		4.68*	

* $P<0.05$ (t test).

that some mechanism for somatic segregation must occur in both the treated and control plants. It must be presumed that the same mechanism is operative for both the single and double spots, and that due to an unequal competitive advantage one genotype may prevail. The more frequent occurrence of the green than the yellow spotting may result from the greater viability of the green cells. The seedlings containing the homozygous AA die in the cotyledon stage as do also the large regions of homozygous AA tissue in leaves.

The similarity of the spots in this tomato mutant to those observed by STERN (1936) in *Drosophila* would seem to indicate that a similar mechanism might be involved. However, the lack of any observation of somatic pairing in plants militates against the acceptance of somatic crossing-over as a predisposing mechanism. A close examination of the somatic chromosomes of this mutant would perhaps give evidence on that point.

It has been suggested to the authors by Dr. G. ÖSTERGREN that a chromatid breakage and interchange between homologous chromosomes, involving the regions containing the *aurea* mutant, would upon later equational division yield two cells — one with the homozygous wild type (++) and the other homozygous for the allele (AA). The extremely frequent occurrence of what would appear on the surface at least to be chance breakage and interchange makes this explanation appear unlikely.

TABLE 5. *Correlation coefficients of number of spots per leaf and size of spot with pollen fertility of colchicine-treated tomato plants.*

Spots per leaf with pollen fertility	+.1288
Size of spots with pollen fertility	—.2165

No significance

The hypothesis of somatic reduction would seem to be supported by these observations, though no critical test to further substantiate it has been carried out. It can, however, be said that a reduction division of at least the region of the chromosome containing this locus has occurred. Whether this takes place as a result of somatic crossing-over, a breakage-interchange mechanism between chromatids of homologues, or by a separation of whole homologous chromosomes must be decided on the basis of further evidence.

The occurrence of the spots, both in the control and treated materials, indicates the normal occurrence in this genotype of the phenomenon giving rise to these spots; but the highly significant difference in the number and size of spots between the treated and untreated plants points to susceptibility of this phenomenon to environmental changes. It is possible that the early treatment with colchicine and infrared light predisposed the cells toward its occurrence, not only more frequently, but at earlier stages of development so that the spots were larger. The phenomenon described here may be similar to that which occurs in sorghum since homozygous tissue appears to be produced in both instances. Within sorghum, however, no naturally occurring homozygous mutants have been observed. This could be due to the infrequent natural occurrence of somatic segregation in sorghum and the difficulty of distinguishing such plants.

SUMMARY

The frequency and size of spots indicating somatic segregation for homozygous tissue in tomato plants heterozygous for the *aurea* mutant were apparently increased by colchicine treatment at the seedling stage. The ratio of green to yellow and also twin to single spots did not differ between the treated and untreated plants. A difference in pollen fertility between treated and untreated plants was noted, probably indicating the occurrence of polyploidy in the treated plants. Correlation coefficients between pollen fertility and frequency and size of spots within the treated group were not significant.

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THE CHROMOSOMES OF TWO CELL STRAINS FROM MAMMARY CARCINOMAS OF THE MOUSE

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I. MATERIALS AND METHODS

IN a search for rat and mouse cell strains suitable for chromosome study, we contacted Dr. KATHERINE K. SANFORD of the National Cancer Institute of the U. S. Public Health Service, who has been successful in adapting mammary carcinomas of the mouse to continuous cultivation *in vitro*. Unfortunately, most of her cell strains had been discarded except two which were still available. One of them contained a feature of specific cytologic interest which stimulated the present report.

The two strains were NCTC 2777 and 2940, derived from mammary carcinomas of C3H female mice. Strain 2777 originated from the transplantable mammary adenocarcinoma C3HBA (BARRETT, DERINGER and DUNN, 1952). This tumor had been carried by Dr. BARRETT for 33 generations in C3H, 7 generations in (C3H×C)F_1, and 146 generations in (C3H·C×C)BC mice prior to its growth *in vitro*. Strain 2940 was a primary tumor received from BARRETT and grown by SANFORD for only two generations in (C3H×C)F_1 mice before its establishment in tissue culture. Culture of Strains 2777 and 2940 was originated on July 16, 1957, and November 26, 1957, respectively. When we received them, therefore, both had a fairly long history *in vitro*, and 2777 in addition a long transplantation history *in vivo*. Pertinent data concerning these lines in tissue culture will be reported by SANFORD.

The two cell strains arrived at our laboratory in June 1959, in two Carrel flasks. The strains were trypsinized and transferred to T-30 flasks in McCoy's 5a medium (MCCOY, MAXWELL and KRUSE, 1959) and later propagated in T-60 flasks and Blake bottles. For chromosome analysis, cells of 2777 were treated overnight with medium containing 1 γ/cc

TABLE 1. *Chromosome numbers in the s-region of the two cell strains.*

Cell strain	2n																	Total cells	Average
	67	68	69	70	71	72	73	74	75	76	77	79	82	83	84	85	86		
2777	1	1	2	9	4	2	9	6	4	1	1	1	—	—	—	—	—	41	73.3
2940	—	—	—	—	—	—	—	—	—	—	—	—	1	5	10	8	1	25	84.1

colchicine. For 2940, such treatment was proven to be too strong. When the treatment was reduced to 6 hours in 0.5 γ/cc of colchicine, the analysis of 2940 could be made, even though some cells still showed heavily contracted chromosomes. Apparently the two lines differed in their susceptibility to colchicine.

II. OBSERVATIONS

Both cell strains were heteroploid with stemline numbers in the tetraploid region of the mouse. As seen from Table 1, 2777 was hypotetraploid with $s=73$, while 2940 was hypertetraploid with $s=84$. Figure 1 gives karyotype analysis of typical metaphase plates of the two strains. Apart from the big biarmed structure in 2777 (Fig. 1 *b*), all the chromosomes were of the telocentric type characteristic of mouse chromosomes. Length measurements would undoubtedly show the extremes of chromosome length to be greater than in the normal mouse karyotype, indicating that random structural variation had been active during the development of the karyotypes of the cell strains (LEVAN, 1956). Cells of Strain 2777 often possessed several minute chromosomes, obviously smaller than any normal mouse chromosome. In general, the karyotypes

TABLE 2. *Chromosome numbers and occurrence of the heterometacentric in 2777.*

Number of HM	2n																	Total cells
	67	68	69	70	71	72	73	74	75	76	77	79	87	93	±2s	±4s	±16s	
0	—	1	—	8	2	2	9	3	—	1	1	—	—	1	4	—	—	32
1	1	—	2	1	2	—	—	3	4	—	—	1	1	—	19	—	—	34
2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	1	—	5
4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1
8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1
Total	1	1	2	9	4	2	9	6	4	1	1	1	1	1	27	2	1	73

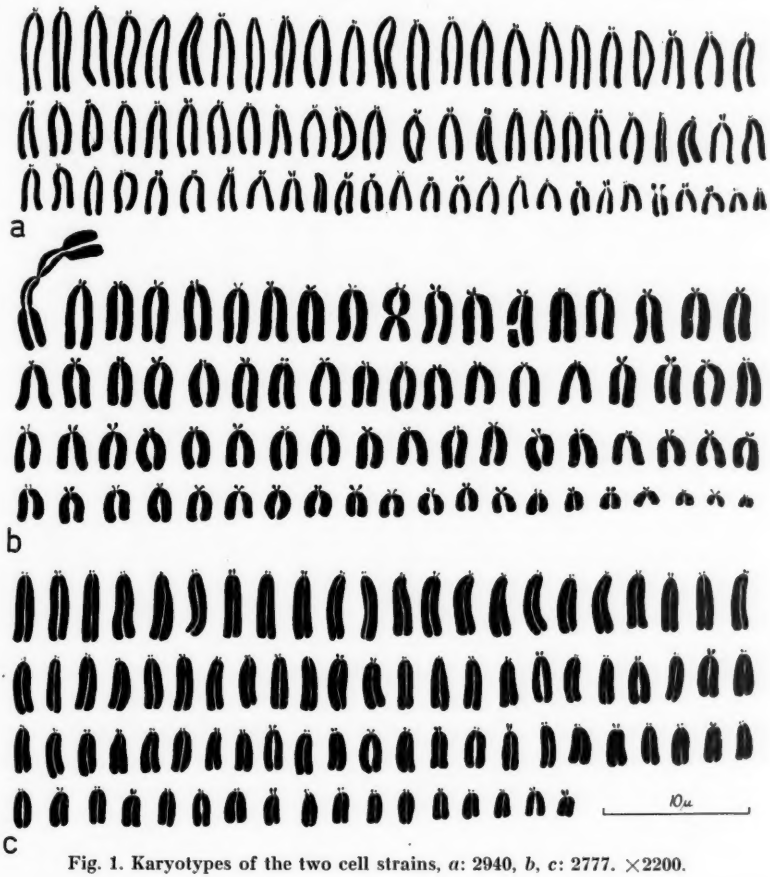

Fig. 1. Karyotypes of the two cell strains, *a*: 2940, *b*, *c*: 2777. ×2200.

agree well with the type of stemline usually found in old mouse tumors, such as the tetraploid Ehrlich or Krebs-2 (HAUSCHKA and LEVAN, 1958).

Of special interest, naturally, was the outstanding metacentric chromosome present in 2777, henceforth referred to as the heterometacentric or, abbreviated, the HM chromosome. It was present in all slides examined through several fixations, and evidently was a permanent member of the stemline of 2777. Among 73 carefully analyzed cells (Table 2), the HM was found in 41 cells. One HM per cell was the rule both in the *s* and in the 2*s* cells; but in the latter category, as in the three cells with still higher numbers, more than one HM could occur. A correla-

tion between the ploidy level and the number of HM's is clearly indicated in Table 2: Among the 41 cells examined on the *s* level, only 14 (34 %) had the HM, while 26 out of 30 cells (87 %) on the 2*s* or higher level had at least one HM. It was rather remarkable that none of the eleven cells in the center of the *s* region, with 72 and 73 chromosomes, showed the HM, which might indicate that a stemline without the HM was under development. With the small number of cells analyzed, however, nothing definite could be concluded. The difference in frequency of HM between the *s* and the 2*s* level, on the other hand, seemed to be well established. Evidently there was a tendency for the HM to be eliminated on the 2*s* level as well as on the *s* level, because most reduplication mechanisms would result in two HM chromosomes per cell in the 2*s* region. The actual observed number per 2*s* cell was 0.96, while the corresponding value for the *s* region was 0.34. This suggests that most 2*s* cells had a history of being at that level, rather than having been recently formed from *s* cells.

The heterometacentric chromosome was a unique cytological structure, a structure that has earlier been observed only as a freak in cancer and tissue culture cells. This was probably the first case in which such a structure was found to be incorporated in the stemline of a cell population. Its appearance may be seen from the drawings of Fig. 2 and the photomicrographs of Fig. 3. In its standard type, the HM was a symmetric structure: Both ends were of the same width as the other chromosomes, while the middle part was considerably narrower. The ends were therefore considered euchromatic, while the middle part was considered heterochromatic and was negatively heteropycnotic. The centromere was located in the center of the heterochromatic section. The terminal euchromatic segments were distinctly set off from the heterochromatin, often by a constriction, and always by a sudden narrowing to the width of the heterochromatin.

The border zones between euchromatin and heterochromatin seemed to be especially sticky. Even after the chromatids had completely separated elsewhere, they might still be held together at these two points in addition to the centromere (Fig. 2 *r*, *s*). The development of the c-pairs of the HM was characteristic: First the chromatids were in contact all along their length (Fig. 2 *a*—*f*); then they touched each other at the centromere and at one or both border zones (Fig. 2 *r*); and eventually they hung together only at the centromere (Fig. 2 *h*—*m*). As they approached telophase, their major spirals might become visible (Fig. 2 *p*, *q*).

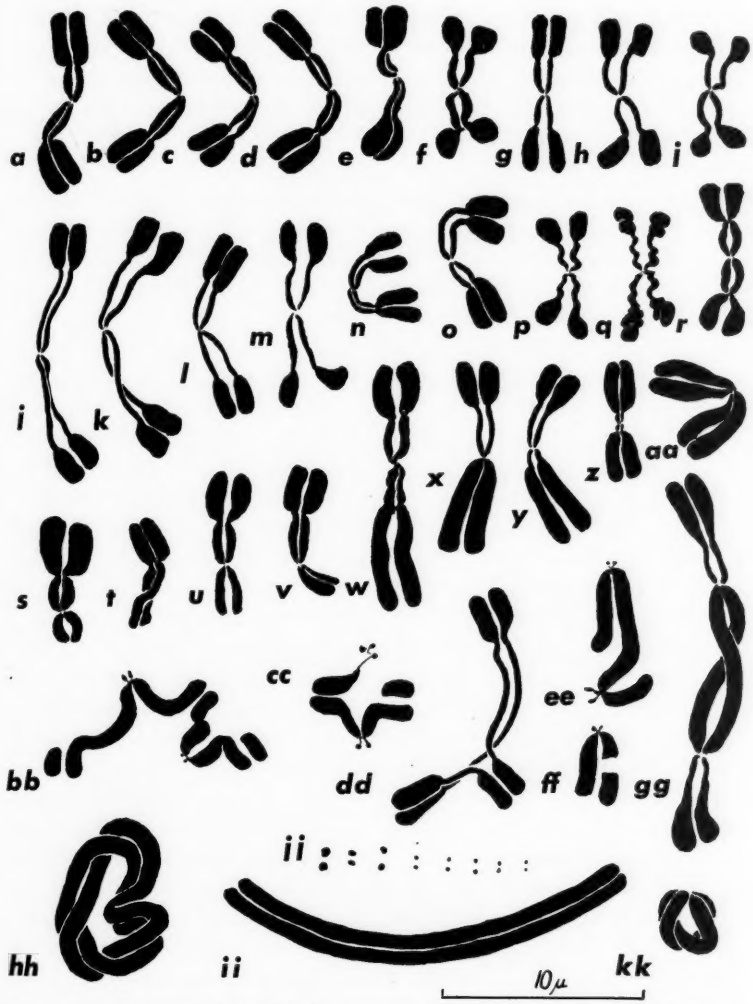

Fig. 2. *a—aa*: instances of the HM chromosome, *bb—kk*: chromosome configurations from cells with high frequency of structural changes. ×3000.

The standard type of the HM was subject to variation. Since chromosome breakages and structural interchanges were fairly frequent in this cell strain, the changes in appearance of the HM were not unexpected. It was somewhat surprising, however, that most of the deviations ob-

served could be recognized as belonging to a few clear-cut types. Possibly these types belonged to different cell lineages; thus, each type occurred in descendants of a cell in which a certain change of the HM had taken place. Alternatively, they might have originated frequently and independently, which would mean that certain points along the HM were more liable to breakages than others.

The types of deviation observed are represented diagrammatically as nos. 2—8 in Fig. 4. No. 2 was the most common type of variation, found 5 times in 56 instances of HM. It differed from the standard type by lacking one of the euchromatic end segments, thus becoming assymmetric (Fig. 2 *s*—*v*, Fig. 3 *c*, *j*). The very long heterochromatic strands of Fig. 2 *i* and *k* (type 3 in Fig. 4) suggest a doubling of the heterochromatic segment. However, a lack of contraction within the heterochromatin would have the same effect. Several observations of unequal heterochromatic segments on the two sides of the centromere speak in favor of structural changes. The cases of assymmetric heterochromatin observed were the following: one case in which the heterochromatin on one side of the centromere was of normal length and the other of approximately double length (Fig. 2 *o* and 3 *e*, Fig. 4 type 4); and three cases in which one side was normal and the other side very short or missing (Fig. 2 *x*, *y*; Fig. 4 type 6). The length of the euchromatic parts of the HM was also variable: The euchromatin might be approximately double the normal length either on one side only (Fig. 2 *w*—*y*, Fig. 4 types 5 and 6), or on both sides (Fig. 2 *aa*, Fig. 4 type 7). A remarkable structure (Fig. 2 *gg*, Fig. 3 *b*, Fig. 4 type 8) was found once in a cell with high chromosome number and a great number of interchanges (Fig. 3 *a*).

Strain 2777 possessed a relatively large fraction of cells with 2*s* and higher numbers. Among cells with high chromosome numbers, the record was held by the one photographically represented in Fig. 3 *a*. This cell exhibited some very interesting features. Its ploidy level was probably 16*s*, that is, $2n = \pm 850$; but a profuse structural reshuffling made the counting of the actual chromosome number impossible. The chromosome elements of this cell ranged in size from 20 μ to 0.1 μ, and probably less. Its largest elements represented fusions of many chromosomes. The long acentric element at the left of the photograph (Fig. 3 *a*), drawn separately on Fig. 2 *ii*, may contain the main bulk of some seven to eight chromosomes of average size. It is suggestive that in the vicinity of this chromosome and several acentrics close by, there was a cloud of numerous tiny fragments, some of which are drawn in

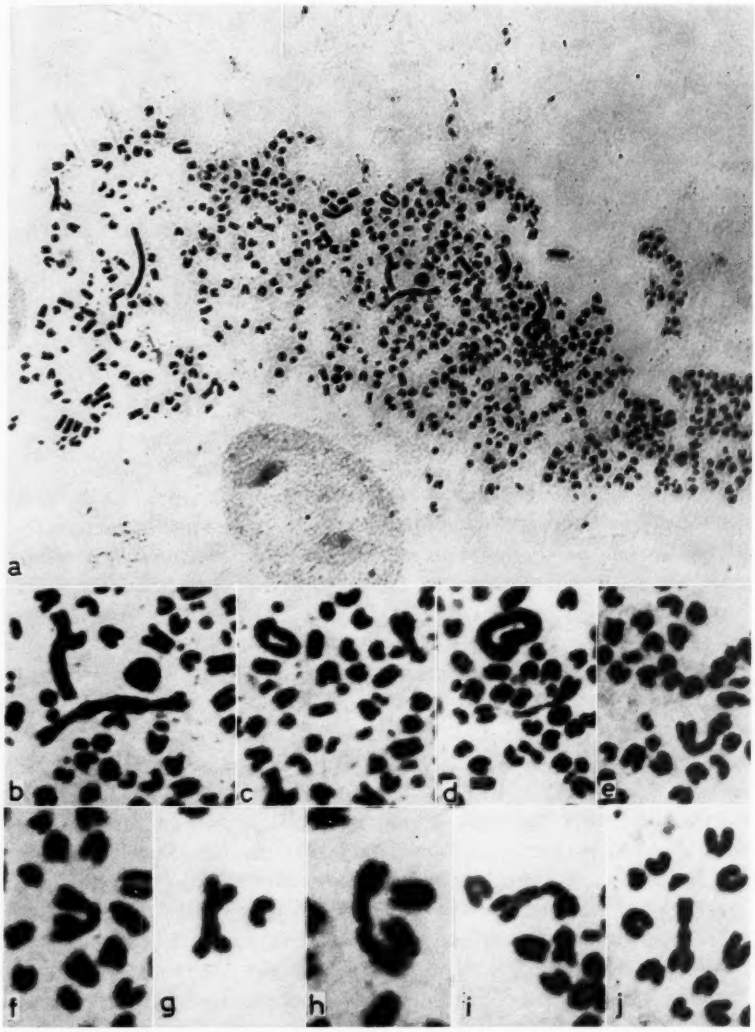

Fig. 3. *a*: cell with high chromosome number and high frequency of structural changes. *b*—*e*: details from *a*. *f*—*j*: instances of the HM chromosome. *a*: ×450, *b*—*j*: ×1200.

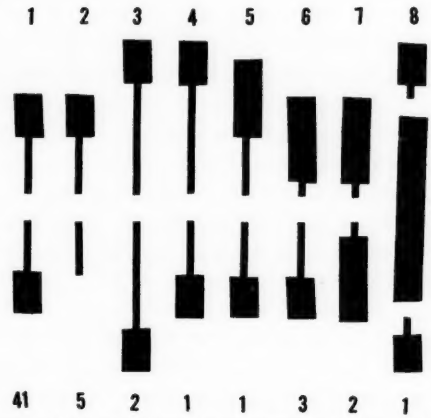

Fig. 4. Diagram of the variations in appearance of the HM chromosome.

Fig. 2 *jj*. All of them consisted of pairs of small bodies, and some may well represent centromeric regions, originally belonging to the acentric elements. A tendency to the formation of long acentric structures has sometimes been noted in tumors and tissue cultures. Such structures were illustrated, for instance, by TJIO and LEVAN (1954, Fig. 10 *m*, *r*, *t*, p. 26), and by LEVAN and BIESELE (1958, Fig. 7, p. 1044). Several ring chromosomes were present in the big cell of Fig. 3 *a*, the largest of which, an acentric, is depicted in Fig. 2 *hh* and Fig. 3 *d*, and another one with a centromere in Fig. 2 *kk*.

Eight heterometacentrics were observed in this cell. Three of them (Fig. 2 *i*, *l*, *o*) appeared normal; two had euchromatin of different lengths in the two arms (Fig. 2 *x*, *y*); one had lost most of its heterochromatin (Fig. 2 *aa*), and the last one had a large section of euchromatin inserted into the middle of the heterochromatin (Fig. 2 *gg*). The last two types differed so profoundly from the standard type that it was difficult to ascertain that they were really derivatives of the HM.

One variant of HM, depicted in Fig. 2 *dd*, is from another cell with many structural changes. In this cell the chromosome number was in the *s* region. A few instances of chromosome rearrangements in this cell are represented in Fig. 2 *bb*, *cc*, *ee*, and *ff*. In Fig. 2 *dd*, the HM chromosome was obviously involved in a structural interchange with one of the normal chromosomes, thus forming a triradial. This element seemed to be acentric.

III. DISCUSSION

The heterometacentric chromosome of Strain 2777 is unique in a number of respects. Even though heterochromatic segments bordering the centromere may be a general feature in chromosomal organization, segments as large as those of the HM are unprecedented. Moreover, the heterochromatin on the sides of the centromere, the so-called proximal or prochromosomic heterochromatin, is normally isopycnotic during metaphase and positively heteropycnotic during interphase. In the present case, it is negatively heteropycnotic at metaphase. The interphase nuclei of 2777 usually contained a great number of heterochromatic bodies that were similar in size, probably corresponding to the centromeric regions of all chromosomes; but contained no strikingly large body that could be identified as the heteropycnotic portion of the HM.

Since both transplantable tumors and tissue culture cell strains have a long history of evolution, it is hardly to be expected that they would still represent the genetic composition of their respective primary tumors. In primary mammary tumors of the viral type, such as those of the C3H strain, usually little chromosomal change can be detected, save the appearance of a heterochromatic element whose origin is obscure (TJIO and ÖSTERGREN, 1958). At any rate, the HM chromosome, or anything similar to it, was not present in the primary tumors studied by TJIO and ÖSTERGREN, or in the other strain (2940) studied by us. Therefore, its development most likely had taken place sometime during the transplantation history of Strain 2777, either *in vivo* or *in vitro*. It would probably not be too far amiss to speculate that the primitive form of HM was an isochromosome, since the most common type was symmetrical in organization and in the proportion of euchromatin and heterochromatin.

Regardless of the origin of the HM chromosome, the cells of 2777 should be useful material for investigations on heterochromatin in higher animals. They should be particularly useful in comparative studies on the chromosomal fine-structure of euchromatic and heterochromatic regions and their zone of transition. Also, they would constitute good material for studying the response of the two types of chromatin to chromosome breaking agents. As pointed out previously, there is some indication that chromosome breakage may be condensed at certain regions of the HM.

The HM would be an excellent marker chromosome in cell population studies. It is suggested from Table 2 that cells without the HM

were the most representative types within the stemline zone. Whether the HM has a tendency to be eliminated from the cell population on account of its awkward shape which handicaps the cells carrying it remains to be investigated. It should not be difficult to test the relative growth vigor of the two types of cells, those with and those without the HM, by using long-term colchicine treatment, a method suggested by HSU and KELLOGG (in press). If cells without the HM proved more vigorous, the recovering population would then contain a higher proportion of cells carrying the HM, since they would tend to remain in interphase during the colchicine treatment and therefore to maintain their original genome, while the more vigorous cells would enter mitosis and be eliminated by acquiring high and imbalanced chromosome numbers.

It would be highly desirable to isolate clones from Strain 2777 so that characterizations could be made for cytologic features, transplantability, *in vitro* growth requirements, and various other functional and cytochemical properties. It would be interesting to elucidate the influence of the HM structure on the evolution of cell populations. One way of doing this would be to combine various proportions of clones lacking and carrying the HM, and to follow the destiny of these experimental populations.

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SUMMARY

Two cell strains, NCTC 2777 and 2940, established in tissue culture from mouse mammary carcinomas, were analyzed as to their chromosomes. Both were heteroploid, with strain 2777 being hypotetraploid, and 2940 being hypertetraploid. A remarkable chromosome, consisting of a long heterochromatic central part where the centromere was located, and two euchromatic end parts, was found in a considerable number of the 2777 cells. Its standard type, and several variants of it, are described and discussed.

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SELF-INCOMPATIBILITY AND OUTCROSSING IN RAPE AND WHITE MUSTARD

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IN order to apply proper methods to the breeding of a specific crop, a thorough knowledge of its mode of fertilization is necessary. When the breeding of oil plants was re-introduced, about twenty years ago, into the breeding programme at Svalöf, it was known that the seed setting of plants, selfed by isolating the inflorescences in bags, was much better in rape (*Brassica napus* L. ssp. *oleifera* (METZG.) SINSK.) than in turnip rape (*B. campestris* L. ssp. *oleifera* (METZG.) SINSK.) and white mustard (*Sinapis alba* L.). Since there was not very much information available regarding the degree of outcrossing or vicinism in these species, studies of this problem were undertaken. Part of this investigation has already been published. The present paper gives a brief summary of some of these results together with some more recent experiences concerning the mode of fertilization in rape.

I. MODE OF FERTILIZATION

1. Rape

The degree of spontaneous crossing between individual plants of rape under normal field conditions has been studied by, among others, SYLVÉN (1920), OLSSON (1952) and PERSSON (1956). In all these studies plants were used which were homozygous for some recessive gene giving a morphological "marker" character. These plants were allowed to flower in large populations with nearly 100 per cent homozygosity for the corresponding dominant gene. The degree of cross-fertilization will show up directly in the progeny of the recessive plants. In the two last cited investigations dark or light yellow colour of the flower was used as marker character since these colour variants were shown to be recessive to normal lemon yellow colour (SYLVÉN, 1927). Besides these cha-

TABLE 1. *The degree of cross-fertilization in individual plants of rape.*

Variety	Percentage of cross-fertilization																				Number of plants	Mean	
	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100			
Local variety	1	1	4	4	3																13	20.2	(SYLVÉN)
Lembke rape	1	1	7	7	10	15	11	4	6	3	4	1	1	—	—	1	—	—	4		76	38.4	(OLSSON)
Svalöf Late rape			1	6	3	9	7	4	3	—	2	—	—	—	1	—	—	—	1		37	37.6	
Sv. Matador rape [1]	3	5	15	11	17	11	5	5	4	—	1	—	—	—	—	—	1	2	5		85	33.3	(PERSSON)
Sv. Matador rape [2]	2	2	11	14	12	12	5	2	2	3	3	1	—	—	—	—	—	1	8		78	37.2	
Summer rape				1	2	—	2	2	1	—	—	—	—	—	—	—	—	—	1		9	42.5	
Total	7	9	38	43	47	47	30	17	16	6	10	2	1	—	1	1	1	3	19		298	35.9	

[1] From a field with a low bee frequency (0.07 bees per m²)
[2] From a field with a high bee frequency (0.39 bees per m²)

racters, SYLVÉN (1920) also used *fasciata* types and differences in the lobation of the leaves and in pod colour.

The results of the studies by SYLVÉN, OLSSON and PERSSON are summarized in Table 1 (from OLSSON and PERSSON, 1958). In rape, on an average, there is about 1/3 cross-fertilization and 2/3 self-fertilization. Corresponding results have been obtained by RIVES (1957). The distribution of the plants in Table 1 is bimodal, with one mode between 20 and 35 per cent of outcrossing, the other at 95—100 per cent. Of the 19 plants around the latter mode, 10 showed 100 per cent outcrossing. In order to verify that this result was not due to a mistake in marking the test plants, the harvest from the progeny plots was sown and the material studied in the following generation as well. In all cases, a typical monohybrid segregation was obtained and it is evident that a population of rape includes plants which are cross-fertilized to 100 per cent. Since the morphology of the flower does not exclude selfing (*cf. e.g.*, PERSSON, 1953; ANDERSSON and OLSSON, 1959), these plants must be self-sterile. This question will be dealt with in a later section of this paper.

The degree of outcrossing between plots in a field is obviously dependent upon the size of the plot, the size of the surrounding field and the degree of coincidence in the time of flowering. In addition, varietal differences may intervene. In small plots, with 3 rows of 2—5 meters and 45 cm between the rows, the degree of vicinism in the author's study varied between 6 and 34 per cent, with an average of 22 per cent. SYLVÉN (1920) and RIVES (1957), however, estimated the degree of intercrossing between neighbouring plots at 0—10 per cent only. The degree of vicinism between two adjacent fields of about the same size decreases very rapidly from the border rows inwards, as shown by TEDIN and NISSEN (1932) in turnip.

Several studies by BECKER (1951), HÄRLE (1951), WAGNER (1954) and PERSSON (1956) show that rape gives a good yield of pods and seeds under enforced selfing. The seed setting in isolation bags is poorer than upon open flowering, however, partly because of an unfavourable micro-climate within the bags, partly because the transport of pollen from anther to stigma is made difficult by the isolation, if artificial self-pollination is not carried out. The general opinion is that the plant can be fertilized by its own pollen and that rape is therefore self-fertile, even if exceptions may occur.

Several rape breeders, *e.g.* SYLVÉN (1926), TROLL (1947), RIVES (1954, 1957), practically completely disregard the risk of inbreeding degenera-

TABLE 2. *The degree of cross-fertilization in individual plants of white mustard.*

Variety	Percentage of cross fertilization									Number of plants	Mean
	92	93	94	95	96	97	98	99	100		
A flower colour mutant	1	—	—	2	1	3	5	19	119	150	99.6

tion in rape. On the other hand, HAGBERG (1953) has shown that even in such a typical self-fertilizer as barley an increase in homozygosity in a cross population results in a decrease in vitality. The experience with swedes shows that evident inbreeding degeneration and F_1 stimulation may occur in this subspecies of *B. napus* (OLSSON, 1936; JOSEFSSON, 1948) and according to recent investigations the same should be true of rape (WAGNER, 1954; MANNER, 1959). Generally speaking, heterozygosity is probably to be preferred to homozygosity, but it is possible to obtain inbred lines which are superior to the mother variety (RIVES, 1957).

2. White mustard

The degree of spontaneous crossing between individual plants of white mustard has been studied with the same methods as used in the case of rape. As test plants, use has been made of three types isolated in X-ray-treated material, *viz.*, 1. a chlorophyll mutant (*luteo-virescens*), 2. a flower colour mutant with nearly white flowers, 3. a mutant with thickened stem and pods. The three characters are all caused by recessive genes and show monofactorial segregation. A detailed study of the degree of cross-fertilization of individual plants has been only carried out with the flower colour mutant. The results are given in Table 2. This mutant as well as the other two has shown practically 100 per cent cross-fertilization.

The degree of vicinism between plots is higher in white mustard than in rape. With plots of 4 rows of 10 meters and 45 cm between the rows, the intercrossing between neighbour plots reached over 30 per cent. In smaller plots, the intercrossing has been even higher (Table 3).

The seed setting after enforced selfing is low, and in the author's experiments only about 20 seeds per 100 isolated flowers have been obtained. This means that about 4 per cent of the ovules have developed into seeds, if, as an average, 5 ovules per pod is calculated. As in, for example, rye (LUNDQVIST, 1958), so are there also in white mustard

TABLE 3. *The degree of vicinism between plots of white mustard.*

	Percentage of vicinism			Mean
	1949	1950	1953	
Plots: 10 m×4 rows				
Border rows	37.3	25.8	41.6	34.9
Inner rows	33.9	19.1	28.5	27.2
Mean	35.6	22.5	35.1	31.1
Plots: 2 m×3 rows				
Border row (row 1)	46.3		50.5	48.4
Inner row (row 2)	36.7		38.8	37.8
Border row (row 3)	44.9		50.9	47.9
Mean	42.6		46.7	44.7

evident differences between plants in a population in regard to the seed setting after selfing.

As usually in normally cross-fertilized species, the forced self-fertilization results in a decrease in plant height and plant weight in the progeny (Table 4). There are marked differences, however, between different inbred lines and some of these were equal or even superior to the mother population. In one inbred family, MANNER (1957) obtained dwarfs, with a height of only 11—14 cm.

3. Some other Brassica species

In brown mustard (*B. juncea* COSS.), turnip rape and black mustard (*B. nigra* KOCH), the degree of spontaneous outcrossing has been determined on a very limited material, and no significant figures are avail-

TABLE 4. *Plant height and plant weight in inbred material of white mustard at Svalöf 1955.*

Material	Number of inbred lines	Plant height		Total plant weight	
		cm	Rel. value	gram	Rel. value
Sv. Primex: Standard		83.6	100.0	35.1	100.0
„ „ I_1-lines	47	80.9	96.8	31.7	90.3
Sv. Primex: Standard		84.7	100.0	32.1	100.0
„ „ I_2-lines	18	79.2	93.5	28.7	89.4
Sv. Primex: Standard		82.8	100.0	35.2	100.0
„ „ I_3-lines	23	75.6	91.3	29.6	84.1

able for these species. The impression is, however, that brown mustard agrees with rape, whereas turnip rape and black mustard give results similar to those of white mustard. This is also true of seed setting in isolation bags and of inbreeding degeneration (MOHAMMAD, 1935; ANDERSSON and OLSSON, 1948; HÄRLE, 1951; WAGNER, 1954).

II. THE INFLUENCE OF THE MODE OF FERTILIZATION UPON THE DEGREE OF HOMOZYGOSITY

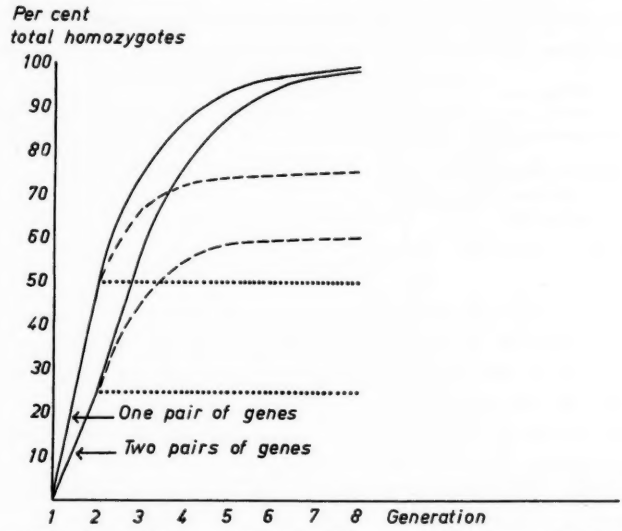
Apart from changes due to selection and mutation within a cross population of a self-fertilizing species, the degree of homozygosity will increase fairly rapidly. A corresponding population of a cross-fertilizer will, however, retain the heterozygosity of F_2 in all following generations. In a partially cross-fertilizing population, a balance will be reached within some generations, and this balance for one pair of genes is easily estimated by the aid of a formula given by MAHBUB and HADLEY (1955).

Starting with an F_1, heterozygous in one pair of genes only (Aa), F_6 of a self-fertilizer will contain 96.875 per cent homozygotes and the value approaches asymptotically towards 100. In cross-fertilizers, the value is fixed at 50 per cent. In a material of rape with $^1/_3$ cross-breeding and $^2/_3$ self-fertilization, F_6 will have 74.69 per cent homozygotes and the value approaches asymptotically towards 75 (Fig. 1). The same level of balance will be obtained, if the homozygotes AA and aa are mixed in equal proportions.

In a material of rape segregating in 2 pairs of factors, the balance is reached at 60 per cent double homozygotes, 30 per cent simple heterozygotes and 10 per cent double heterozygotes, and this level is practically attained in F_7. In the same generation, a self-fertilizer contains 96.9 per cent double homozygotes, whereas in a totally cross-fertilizing population there are 25 per cent double homozygotes, 50 per cent simple heterozygotes and 25 per cent double heterozygotes. If, in the rape material, there is segregation in a large number of genes, the frequency of total homozygotes is very low, and considering the large number of genes varying in a rape population complete homozygotes are most probably exceedingly rare.

III. SELECTION FOR SELF-INCOMPATIBILITY IN RAPE

As already pointed out (page 243), there occur plants in a common rape variety which are self-sterile. In order to verify this observation



——— Complete selfing; – – – $^2/_3$ of selfing; ..... Total cross fertilization.

Fig. 1. Curves for the degree of homozygosity in one and two pairs of genes at different levels of cross- and self-fertilization.

and in an attempt to subdivide a market variety into two populations, one with self-compatible and mainly self-fertilizing plants, the other with self-incompatible and cross-fertilizing plants, selection for the degree of self-incompatibility has been carried out in Svalöf's Matador winter rape.

The work has included a study of the ability of the pollen to germinate and to penetrate the stigmatic surface upon self-pollination and also a determination of the seed setting after bag isolation. For a study of the pollen tube growth, the stigma and style were cut lengthwise with a razor, about 24 hours after pollination, into 3—4 sections which were coloured with 0.1 per cent Cotton blue (DARLINGTON and LA COUR, 1942). The slides were kept over night in an incubator at 50—60° C, and the tissues were somewhat pressed before being studied. By this method it was easy to study the behaviour of the pollen tubes in the superficial layers of the stigma but it was not possible to follow them down through the style.

In 1958 the ability of the pollen to grow on the stigma after selfing was studied on 515 plants of Matador winter rape. In 31 of these plants

TABLE 5. *Distribution of families in different compatibility classes.*

Material	Growing ability of pollen in selfing				Number of families
	1.0—1.9	2.0—2.9	3.0—3.9	4.0—5.0	
Families from self-compatible mother plants	—	5	6	10	21
Families from self-incompatible mother plants	15	4	2	—	21

the growth of the pollen tubes was evidently inhibited. Bag isolations were then made on these plants as well as on a corresponding number of plants where the pollen after selfing had clearly grown into the stigma. In the group with self-compatible plants there were 4.14 ± 0.47 seeds per flower, in the group with self-incompatible plants there were only 0.94 ± 0.33 seeds per flower. The seed setting was on the whole low, but the difference between the two groups is fully significant ($t = 5.57^{***}$).

The following year it was possible to investigate progenies from 21 plants of each group. The material was grown in two separate chambers of a greenhouse and the ability of the pollen to grow in the stigma after self-pollination was determined on 10 plants in each family. The growing ability of the pollen was graded, plants with no pollen tubes penetrating the stigmatic surface, were graded with 1, whereas plants where a large portion of the pollen had penetrated into the style were given 5. The distribution of the families in different classes of self-compatibility is given in Table 5.

The difference between the two groups is quite evident. There are some families originating from self-compatible mother plants, however, where the pollen growth is evidently inhibited, or where an evident segregation of self-incompatible individuals has taken place (Table 6). A few mother plants, quoted as self-incompatible, have given a fairly self-compatible progeny.

The main aim was to select one self-fertile and one self-sterile population. After determination of the growing ability of the pollen tubes all predominantly self-incompatible plants were, therefore, discarded in the former group, and all predominantly self-compatible plants in the latter. On each of the remaining plants, as a rule 30 buds were isolated in bags. The seed setting in the bags was obviously much better in the self-fertile than in the self-sterile group (Table 7). Ten families originat-

TABLE 6. *The degree of growth of the pollen tubes and the seed setting after selfing in one self-compatible, one self-incompatible and one segregating family of rape.*

Plant no.	Self-compatible		Segregating family		Self-incompatible family	
	Pollen tube growth	Seeds per flower	Pollen tube growth	Seeds per flower	Pollen tube growth	Seeds per flower
1	5	8.7	5	9.3	1	0.10
2	5	12.7	1	0.1	1	0.03
3	5	5.6	5	8.2	1	0.23
4	4	6.1	5	10.6	1	0.00
5	5	6.7	4	11.2	1	0.00
6	4	15.2	5	13.3	1	0.10
7	5	4.9	4	14.7	1	0.00
8	5	6.2	1	0.1	1	0.00
9	5	7.4	4	11.4	1	0.00
10	5	6.5	5	14.3	1	0.03
Mean	4.8	8.0	3.9	9.3	1.0	0.05

ing from self-incompatible mother plants had not more than 0.1 seeds or less per isolated flower and at the same time the growth of the pollen tubes was strongly inhibited. These plants no doubt are as strongly self-incompatible as the plants in *e.g.*, a population of white mustard.

Another significant observation is that, upon open flowering in greenhouses with insufficient supply of pollinating insects, the seed setting is only 11.3 ± 0.58 per flower in the self-sterile group, but 21.0 ± 0.41 in the self-fertile group. After artificial crossing, there were no differences between the groups with a seed setting of 17.6 ± 1.7 and 16.0 ± 2.2 seeds per flower, respectively.

The experiments are still in progress; so far, however, they have shown that selection for self-incompatibility in rape may result in a

TABLE 7. *Seed setting in isolation bags of different families of self-compatible and self-incompatible rape.*

Material	Number of families	Number of seeds per isolated flower			Mean
		< 1.0	1.0–3.0	> 3.0	
Families from self-compatible mother plants	18	—	—	18	6.32
Families from self-incompatible mother plants	18	13	4	1	0.68

population with complete cross-fertilization. According to the annual reports (1957 and 1959) of the Scottish Plant Breeding Station, a self-incompatible material has been found in the breeding material of swedes at this station, a material which is now being subjected to closer study. Such self-sterile strains should be of value in the breeding of rape and swedes, since they make is possible to utilize the effect of heterosis.

IV. DISCUSSION AND SUMMARY

The flowers of the *Brassica* and *Sinapis* species dealt with here are morphologically very similar, and self-pollination as well as cross-pollination are possible. When the anthers open, those of the long stamens are about on a level with the conceptive stigma. In most cases the anthers turn somewhat outwards, which renders self-pollination more difficult without preventing it. On the other hand, the morphology of the flower does not completely guarantee a 100 per cent selfing, which may be clearly demonstrated if the plants are grown in greenhouses, free from insects and protected against wind (HÄRLE, 1948). In all the species dealt with, the flowers are fairly large, attractive to insects and with abundant nectar. They are very frequently visited by pollinating insects such as bees and bumble bees, and these no doubt contribute to an increase in cross-fertilization although their importance for the seed setting, at least in rape, must not be overrated (*cf.* MEYERHOFF, 1954; PERSSON, 1956). In openly flowering material the wind also plays an important role in pollen transportation (OLSSON, 1955).

The difference shown to exist here among, on the one hand, white mustard, turnip rape and black mustard, which are practically completely allogamous, and, on the other, rape and brown mustard, which are selfed to about $^2/_3$, is not caused by differences in the morphology of the flowers but by differences in self-compatibility.

Since the species with higher chromosome numbers are the ones with a high degree of self-compatibility, WAGNER (1954) and others are of the opinion that the self-compatibility is caused by the amphidiploidy as such. According to HOWARD (1942), however, in *Brassica campestris* and in *Raphanus sativus* autotetraploid as well as diploid branches of chimaeras, obtained after colchicine treatment, have been self-incompatible. Also autotetraploids of *B. campestris* and *B. nigra*, produced by RAMANUJAM and DESHMUK (1945), were self-incompatible. In the present author's earlier studies of artificial rape (OLSSON, 1953), some plants were self-compatible, others completely self-incompatible. The

conclusion was then drawn that the allopolyploidy as such does not result in self-compatibility, but that this must instead be caused by specific gene combinations. This opinion is strengthened by the fact reported here, that it is possible within a population of natural rape to select self-fertile and self-sterile sub-populations, which are both obviously amphidiploid. Demonstrating incompatibility with sporophytic determination within *Cruciferae*, BATEMAN (1955) has shown that polyploidy alone does not result in self-fertility.

The selection of self-sterile strains will make it possible to utilize heterosis effects in rape breeding. This possibility may be still further developed by the use of male sterile strains. TOKOMASU (1951, 1957) has thoroughly studied such types in radish, and male sterility seems also to occur in rape (KOCH and PETERS, 1953, and the author's own observations).

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TRANSMISSION HÉRÉDITAIRE DES LÉSIONS CHROMOSOMIQUES DUES À LA 8-ETHOXYCAFÉINE

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DANS différentes recherches, KIHLMAN (1949, 1950, 1951, 1952) a montré l'activité pharmacodynamique des dérivés caféiniques substitués sur le carbone 8 dans la production d'accidents radiomimétiques. Chez *Hordeum sativum*, EHRENBERG, GUSTAFSSON et von WETTSTEIN (1956) ont démontré que la 8-Ethoxycaféine accroît fortement la stérilité en première génération sans augmenter notablement le taux de mutations. On serait donc en présence d'une substance capable de casser les chromosomes sans induire de mutations visibles.

L'étude des effets de la 8-Ethoxycaféine a été reprise chez *Hordeum sativum* et chez *Vicia faba* par J. et M. MOUTSCHEN-DAHMEN (1958). La spécificité d'action de cette substance a été observée dans les méristèmes radiculaires pendant toute la période durant laquelle des cassures chromosomiques apparaissent chez les plantes traitées. Les cassures ne se produisent pas au hasard mais sont localisées en des zones bien précises du génome. Chez *Vicia*, une proportion importante de cassures a lieu au niveau de la constriction nucléolaire et aussi au niveau des constrictions centromériques. Etant donné l'existence de ces cassures en des endroits spécifiques durant une très longue période du développement radiculaire, nous nous sommes proposé de voir si certaines de ces lésions persistent durant le développement de toute la génération traitée ainsi qu'à la génération ultérieure.

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I. LES LÉSIONS À LA GÉNÉRATION TRAITÉE (X_1)

Dans un précédent travail, nous avons montré que des cassures chromosomiques sont susceptibles de se produire dans les méristèmes radiculaires après des périodes très éloignées du moment de traitement. Il s'agit, dans ces cas, de cassures chromosomiques secondaires. Ces cassures se produisent à un niveau électif caractéristique de l'action de cette substance et qui est probablement situé au point de jonction du satellite et de la constriction nucléolaire.

Ce phénomène est-il propre aux tissus radiculaires? Nous pouvons actuellement répondre qu'il est constant dans tous les tissus. Nos investigations cytologiques ont porté sur des primordia foliaires et floraux et sur les tissus sporogènes des anthères provenant de 30 plantes.

Le Tableau 1 indique un maintien des lésions jusqu'au moment de la meïose. Tous les individus examinés sont affectés. Le taux de lésions: cassures, déficiences chromosomiques et réunions est néanmoins, en général, plus élevé dans les primordia que dans les tissus prémeïotiques.

Il semble donc que l'on puisse conclure qu'il y a élimination de lésions au cours de la genèse de ces organes. Qualitativement, nous avons retrouvé tous les types de lésions chromosomiques tardives décrites dans les méristèmes radiculaires: configurations polyradiales néanmoins fort rares dont la localisation s'est révélée dépendre dans tous les cas observés, de la spécificité d'action de la substance; cassures localisées dans la zone de constriction nucléolaire et les zones centromériques; 10 cas de «chromosomes fourchus» (pour 30 plantes). Deux de ces cas consistaient en une réunion du satellite près ou dans la zone centromérique

TABLEAU 1. *Types de lésions dans différents tissus (nombres par cellule).*

Types de tissus	Lésions observées						Nbre de cell. exam.
	Cassures chromosomiques	Chromosome M privé de satellite	Micronoyaux	Ponts anaphasiques	Polysomiques	Aneuploïdes	
Primordia foliaires	0,041	0,052	0,068	0,064	0,042	0,010	1000
Primordia floraux	0,042	0,051	0,024	0,052	0,032	0,010	1000
Tissus prémeïotiques	0,021	0,041	0,010	0,031	0,020	0,005	1000

TABLEAU 2. *Différentes anomalies meïotiques observées.*
(1000 figures appartenant à 30 plantes)

Stade meïotique observé	Perte d'un chromos. S	Gain d'un chromos. S	Configurations polyradiales	Dicentriques ou ponts	Fragments chromos.	% de figures perturbées
Diakinèse et métaphase	0,032	0,080	0,030	0,062	0,015	21,8
Anaphase I	0,035	0,039	0,088	0,259	0,018	20,2
Anaphase II	—	0,002	—	0,012	0,013	4,3

du chromosome M. Cette figure n'avait pas été observée dans les méristèmes radiculaires. LEVAN (1932) rapporte un cas d'attachement d'un fragment chromosomique près du centromère chez *Allium allegheniense*. GOODSPEED et AVERY (1930) ont décrit dans le genre *Nicotiana* un génome similaire.

Alors que les lésions caractéristiques de la 8-Ethoxycaféine se retrouvent chez tous les individus étudiés avant la meïose, seuls 22 individus ont révélé des perturbations meïotiques; chez les individus restants, aucune anomalie n'a pu être détectée. Dans un lot témoin, le taux de lésions ne dépasse jamais 0.001 sur plusieurs milliers de cellules examinées. Le Tableau 2 résume les observations des anomalies meïotiques.

Les anomalies meïotiques observées sont assez nombreuses et souvent assez compliquées. De la même manière qu'avant la meïose, tous les types d'anomalies primitivement rencontrées sont susceptibles d'être retrouvés.

Lorsque l'on compare les Tableaux 1 et 2, on voit que le taux d'aberrations augmente en meïose au lieu de diminuer comme l'évolution générale des lésions pouvait le laisser prévoir. On peut dès lors, se demander s'il ne s'agit pas d'une néogenèse de lésions apparaissant au moment où les conditions physiologiques changent d'une manière importante.

L'accroissement des taux de cassures et surtout de réunions en anaphase I par rapport à la métaphase étaye ces vues. Dès ce moment, on se pose la question de savoir quelle est la possibilité de transmission de ces lésions en X_2. Le nombre très restreint d'aberrations observées en anaphase II semble bien montrer une élimination rapide et fortement agissante des lésions antérieurement relevées.

II. LES LÉSIONS À LA GÉNÉRATION ULTÉRIEURE AU TRAITEMENT (X_3)

Les 30 individus de la génération antérieure ont été autofécondés. 90 plantes obtenues ont été analysées cytologiquement. Des lésions sont observées dans les méristèmes radiculaires lors de la seconde mitose après la germination de la graine. 43,3 % des plantes examinées montrent des lésions chromosomiques. Ces lésions chromosomiques consistent essentiellement en cassures et en réunions. Les lésions complexes décrites à la génération précédente n'ont pas été observées. Jusqu'à présent, il n'a pas été possible d'isoler une souche aneuploïde ou polysomique. Les lésions observées dans les méristèmes radiculaires à cette génération se comportent comme celles des méristèmes radiculaires des parents.

Des graines provenant d'une seule plante ne sont pas caractérisées par un caryotype commun. Les fréquences des lésions observées (à la seconde mitose après germination de la graine) sont variables d'une plante à l'autre comme l'indique le Tableau 3.

Toutes les plantes analysées présentent néanmoins des caractères communs dans la répartition des cassures chromosomiques dans le génome. Les cassures chromosomiques sont loin d'être réparties au hasard.

Pour l'ensemble des investigations cytologiques portant sur cette génération, nous avons obtenu la répartition suivante (en seconde mitose après la germination pour 600 figures mitotiques environ):

% des cassures chromosomiques dans le génome						
Bras nucléolaire	*Chromosome M*				*Chromosome S*		
	Satellite	1,6	51,3	68,6	Régions non centromériques	25,4	31,4
	Constriction nucléolaire	19,9			Centromères	6	
	Segment distal	29,8					
	Centromère	10,4					
	Bras non nucléolaire		6,9				

Dans le cas de la race qui nous occupe, le rapport $\frac{\text{n. de cassures S}}{\text{n. de cassures M}} = 2{,}37$ lorsque les cassures sont réparties au hasard.

Ici, on note un rapport $\frac{\text{n. de cassures S}}{\text{n. de cassures M}} = 0{,}46$.

Il y a donc un gros excédent de cassures localisées dans le chromosome M et dans celui-ci, une très forte proportion est concentrée dans

TABLEAU 3. *Fréquences des lésions observées dans 6 graines X_2 provenant d'une seule plante autofécondée X_1.*

Lésions	Plantes					
	I	II	III	IV	V	VI
% d'anaphases perturbées	28,6	1	47	3	25,2	32,3
% de métaphases perturbées	16	—	46	16,6	26,1	44
N. de fragments chromos. par cellule (500 cell.)	0,257	0,01	0,540	0,01	0,20	0,36
N. de ponts par anaphase (500 cell.)	0,06	—	0,192	0,02	0,05	0,04

le bras nucléolaire. L'examen des figures mitotiques anormales a révélé qu'au moins la moitié des cassures de ce bras était localisée dans ou contre une zone hétérochromatique juxta-centromérique.

Nous nous trouvons ici en présence d'une différence significative de répartition des cassures avec les plantes de la génération traitée. A la génération traitée, cette localisation n'existe pas mais on observe d'autre part, une proportion de loin supérieure de satellites isolés. Nous n'avons jamais remarqué une telle systématisation des cassures dans le cas des contrôles.

Concernant l'évolution du taux de ponts chromosomiques, celle-ci est fort différente d'un individu à l'autre. Le Fig. 1 montre que, dans certains cas, l'élimination au cours du développement est beaucoup plus rapide que dans d'autres. Chez d'autres individus, au contraire (Fig. 2), il n'y a pas élimination mais accumulation de ponts. Dans ces derniers cas, nous n'avons pas constaté la présence de nouveaux fragments chromosomiques et il est fort probable que nous avons affaire à un cycle: cassure-pont-cassure, tel par exemple, celui décrit par McCLINTOCK (1941) chez *Zea mays* et par KOLLER dans les cellules tumorales du rat (1953).

On est donc amené à postuler que

1') d'une part, au début de l'évolution des méristèmes radiculaires en X_2, il y a néogenèse de lésions;

2') d'autre part, que cette production de nouvelles lésions disparaît et que les lésions résiduelles observées ultérieurement n'apparaissent que comme la conséquence mécanique des lésions premières.

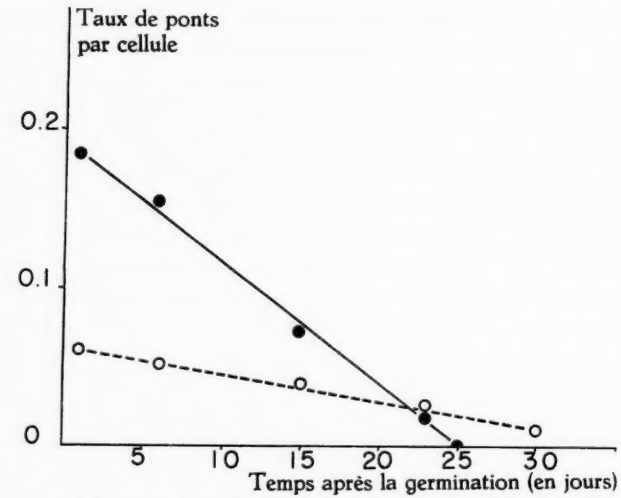

Fig. 1. Diminution du taux de ponts par cellule au cours du développement dans le cas d'individus différents.

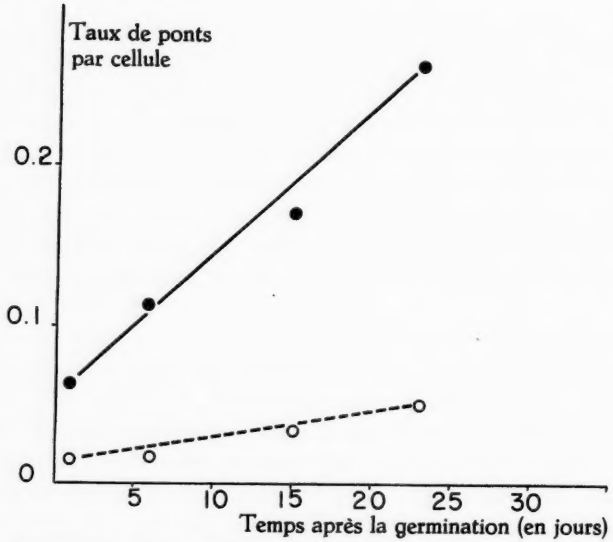

Fig. 2. Augmentation du taux de ponts par cellule au cours du développement dans le cas d'individus différents.

III. MANQUE DE PARALLÉLISME ENTRE LÉSIONS CHROMOSOMIQUES ET MORPHOLOGIE DES PLANTES

Une diminution de fécondité a été observée à la génération parentale: 14,5 % des plantes sont stériles (contre 2,1 % dans un lot non traité) et environ 10 % de fleurs sont stériles, pour l'ensemble des 30 plantes examinées. Une telle modification de fécondité n'a cependant pas été observée à la génération utérieure. A la dose utilisée dans ces expériences, il n'existe pas de modifications significatives du pouvoir germinatif des graines.

Aucune mutation morphologique drastique affectant l'un ou l'autre stade du développement de la plante n'a été constatée. On observe des différences dans la vitalité des individus X_1 ou X_2 mais aucune relation n'a jusqu'à présent, pu être établie entre la réduction de vitalité des plantes et l'intensité des lésions chromosomiques ainsi que leur maintien pendant une période plus ou moins longue. Les seules modifications observées en X_2 consistent en une plus grande variabilité de la morphologie foliaire ou florale. Un type morphologique est cependant constant chez un même individu.

CONCLUSIONS

1) L'examen des lésions chromosomiques dues à la 8-Ethoxycaféine à différentes périodes du développement de *Vicia faba* montre le maintien jusqu'à la meïose de ces lésions et de leurs localisations spécifiques dans le génome.
2) Des aberrations chromosomiques se transmettent dans un grand nombre d'individus à la génération ultérieure.
3) A cette génération, les modifications des taux de cassures et de réunions chromosomiques ainsi que les changements profonds de leur répartition dans le génome plaident en faveur d'une néogenèse des lésions.
4) Il n'y a pas de parallélisme entre l'intensité des lésions chromosomiques et les modifications phénotypiques d'ailleurs fort peu profondes, opposant l'action du toxique à celle d'autres drogues mutagènes.

Nous nous faisons un plaisir d'exprimer tous nos remerciements à Monsieur le Professeur Å. GUSTAFSSON pour son aide précieuse. Nous remercions aussi Mr le Dr L. EHRENBERG pour ses nombreuses et

fructueuses suggestions et Mr le Dr B. KIHLMAN qui a aimablement mis à notre disposition la quantité d'éthoxycaféine nécessaire aux expériences.

SUMMARY

Occurrence during two successive generations of chromosome damage caused by 8-Ethoxycaffeine in Vicia faba

30 plants of *Vicia faba* treated with E.O.C. were scored for chromosome breaks. From these 30 plants, 90 daughter plants were obtained after selfing, in which root tips showed chromosome lesions. The distribution of the breaks in the genome was compared with that of the parents. Some modifications were reported but some specificity of the compound was maintained. The progeny plants showed a very low amount of morphological abnormalities in spite of a high rate of chromosome disturbances.

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RADIATION RESPONSE OF SOAKED BARLEY SEEDS

II. RELATION OF RADIOBIOLOGICAL DAMAGE TO SUBSTANCES LOST BY LEACHING[1]

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I. INTRODUCTION

IT has been known for a number of years (cf. GUSTAFSSON, 1947; PATT, 1953) that soaked seeds are damaged more by X-rays than non-soaked seeds. From most of these studies it was concluded that as tissues increased in water content their sensitivity to X-rays also increased. Recently CALDECOTT (1955, a and b) and CURTIS *et al.* (1958) have indicated that seeds with very low moisture levels are more sensitive to X-rays. This suggests that something other than moisture *per se* may be responsible for increased X-ray injury of soaked seeds.

Experiments have been conducted to determine whether water *per se* or chemical and physiological changes induced prior to X-irradiation by water during soaking were responsible for changes in the response of seeds to X-rays. Water in which the seeds were soaked was analysed to determine the nature and amounts of organic and inorganic substances lost from the seeds (KAMRA *et al.*, 1960). The present study is an attempt to ascertain whether the biological damage in the seeds is related to the loss of these substances during soaking.

[1] This paper constitutes part of the thesis submitted by the senior author to the Graduate School of the Washington State University in partial fulfillment of the requirements for the Ph. D. degree in Genetics. Scientific paper number *1865*, Washington Agricultural Experiment Stations, Pullman. Work was conducted under projects 1002 and 1068, and supported in part by U. S. Atomic Energy Commission No. AT (45-1)-353 and funds provided for medical and biological research by the State of Washington Initiative Measure 171.

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II. MATERIAL AND METHODS

Barley (*Hordeum vulgare*, hulless variety Himalaya CI 620) seeds (caryopses) were used in this study. Spikes were threshed by hand and only unbroken, fully-formed seeds with 8 per cent moisture (attained by storing in desiccators over dry calcium chloride) were used.

1. Soaking treatments

The seeds were soaked for different time intervals (0, 2, 4, 6, 8, 10, 12, 20, and 24 hours) at 20° and 30° C while different gases (nitrogen or oxygen) were continuously bubbled through the water (KAMRA *et al.*, 1960). Control seeds were either not soaked or soaked in oxygen-free distilled water (pH approximately 5.8), which was boiled for at least 15 minutes and then cooled with nitrogen gas bubbling through it.

The gases used in these experiments were obtained from commercially filled cylinders. Analysis of these was as follows:

Oxygen = 99.6 % O_2 + 0.4 % N_2
Nitrogen (water pumped) = 99.6 % N_2 + 0.4 % O_2 and CO_2.

After soaking, the seeds were quickly dried with fan-forced air. When air dry, the seeds were placed over a saturated solution of KNO_3 and NH_4Cl in closed containers to bring the moisture content of the seeds to approximately 15 per cent before X-radiation.

2. X-radiation of seeds

The seeds, embryos facing up, were placed in a single layer in petri dishes. The dishes were centered on a turn table situated 42 cms. from a beryllium window A.E.G. 50 Machlett X-ray tube operated at 34 kv and 25 ma. A thin Saran plastic film was draped over the dishes during X-radiation to reduce moisture changes in the seeds. These conditions provided 800 r/min. of soft X-rays (unfiltered) at the surface of the seed. Since the seeds were at 15 per cent moisture and hydrated immediately after exposure, the after effects of radiation are minimized (CALDECOTT, 1955 a).

3. Effects measured

The influences of the soaking treatments on X-ray sensitivity of seeds were measured by (A) height of M_1 seedlings (B) frequencies of chromosomal aberrations in shoot tips of M_1 seeds and frequencies of seedling chlorophyll mutation in M_2 populations.

A. *Relation of seed soaking to X-ray induced injury of M_1 seedlings*

Fifty seeds were used for each dosage. Following X-radiation the seeds were sown on blotters moistened with distilled water in petri dishes. After one week's growth in germinators with constant illumination at 20° C the resulting seedlings were measured. Growth was recorded as length in centimeters from the base to the tip of the leaf. When treatments produced an effect such that the leaf did not emerge from the coleoptile sheath, the measurements included only the tip of the leaf and not the coleoptile sheath.

B. *Relation of seed soaking to X-ray induced chromosome aberrations and chlorophyll mutations*

A limited number of treatments were selected for cytological and mutation studies following seedling height experiments. Those treatments that produced week-old seedlings at least 5 centimeters long were selected for the mutation test. Experience had shown a high probability of survival in field plantings for M_1 seedlings of this size.

For the cytological and mutation studies, 1050 seeds were soaked in 75 ml. of oxygen-free distilled water for each time of treatment. In the nitrogen soakings, time intervals were 0, 6, 12, 16, 20 and 24 hours. Each was exposed to 0, 10, 15 and 30 Kr of X-radiation. The time intervals were 0, 3, 6, 9, 12, 15, 18 and 24 hours for oxygen soaking and each was exposed to 0, 10, 15 and 20 Kr of X-rays. As in seedling height tests, all seeds were quickly dried after soaking, then held for 5 days over saturated $KNO_3 + NH_4Cl$ in closed containers. Soaked seeds were divided into four equal lots and X-rayed separately as four replications. Following irradiation the seeds were planted on moist blotters in petri dishes and allowed to germinate in the dark at 20° C.

After 24 to 40 hours of germination, 50 seeds of each of the above treatments were fixed in Carnoy's 4 : 3 : 1. The time when the shoot tips of the seeds contained cells in the first mitotic division varied with the treatments. The acetocarmine smear technique of SMITH (1947) was employed in the cytological examinations. Frequencies of dicentric bridges and acentric fragments (rods, dots, isodiametrics) were recorded at mid-anaphase of the first division from every treatment.

After three days of germination, the remainder of the sprouted M_1 seeds were hand planted 2—3 inches apart in the field. All of the mature spikes from individual M_1 plants were harvested and kept in separate envelopes. Mutations among the M_2 progenies were recorded during the

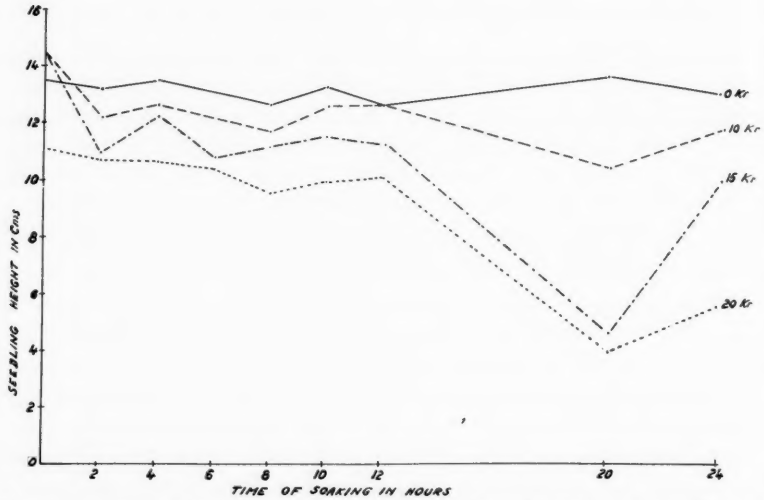

Fig. 1. Average height of one-week old seedlings from X-rayed and non-X-rayed barley seeds soaked in water bubbled with nitrogen for different periods of time at 20° C and dried back to 15 per cent moisture before irradiation. Seedlings grown at 20° C.

late fall and winter from greenhouse plantings of unthreshed M_1 spikes after the method of STADLER (1928). In recording chlorophyll mutations, the designations of GUSTAFSSON (1940) were followed. Other mutations included the fused or onion leaf and elongated or vine seedlings. The temperature in the greenhouse varied from 75° F to 95° F during the experimental period. All of the treatments in a replication were grown at the same time in one greenhouse to avoid effects of light and temperature change.

III. EXPERIMENTAL RESULTS

1. Relation of seed soaking to height of M_1 seedlings

The seedling height from nitrogen soaked seeds at 30° C and grown at 20° C after X-radiation (Fig. 1) was less than from seeds soaked at 20° C (Fig. 2). This was very striking in oxygen soakings (not shown in the figures) where seed germination was greatly enhanced.

Oxygen soaked seeds at both 20° and 30° C were more severely injured by X-rays than nitrogen soaked seeds. X-ray induced seedling

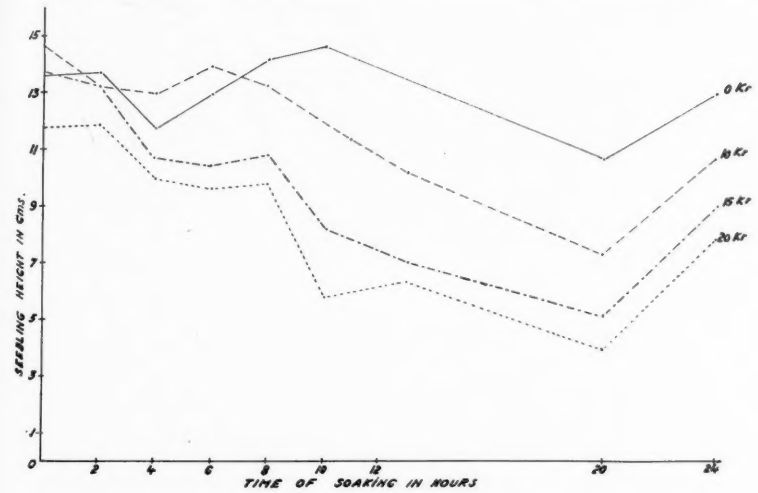

Fig. 2. Average height of one-week old seedlings from X-rayed and non-X-rayed seeds soaked in water bubbled with nitrogen for different periods of time at 30° C and dried back to 15 per cent moisture before irradiation. Seedlings grown at 20° C.

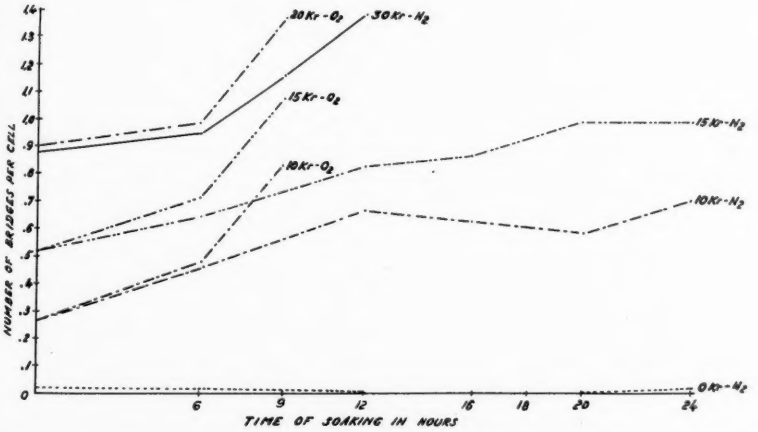

Fig. 3. Average number of dicentric bridges from shoot tip cells of X-rayed and non-X-rayed barley seeds, soaked in water bubbled with nitrogen or oxygen for different periods of time at 20° C and dried back to 15 per cent moisture before irradiation (average of four replications).

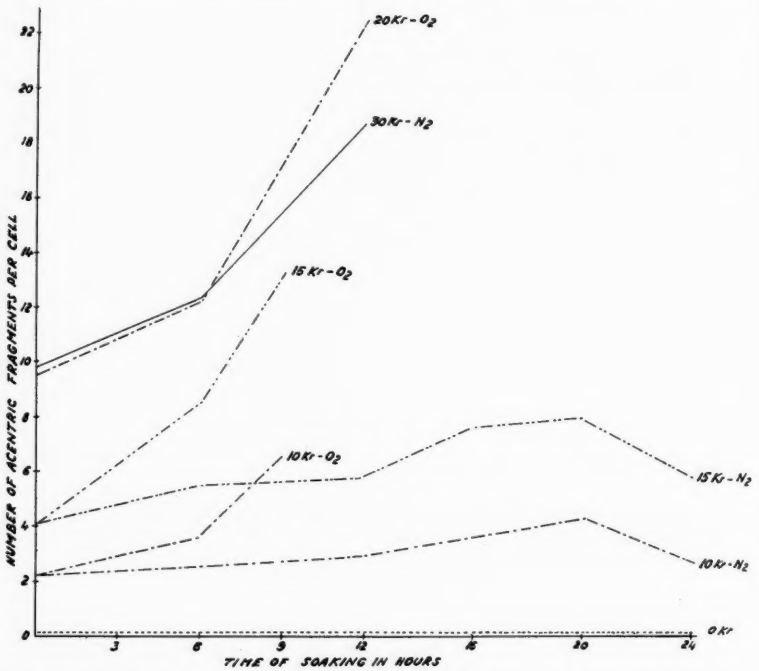

Fig. 4. Average number of acentric fragments (rods, isodiametrics and dots) from X-rayed and non-X-rayed barley seeds soaked in water bubbled with nitrogen or oxygen for different periods of time at 20° C and dried back to 15 per cent moisture before irradiation (average of four replications).

damage increased strikingly after 8—10 hours of soaking and was related to the time of soaking, *i. e.*, the longer the soaking the shorter the seedling height (Figs. 1 and 2).

Nitrogen soakings for 20 hours resulted in greater radiation damage than shorter (12 hours or less) or longer (24 hours) treatments (Figs. 1 and 2).

2. Relation of seed soaking to chromosome aberrations in shoot tip cells of M_1 seeds and to seedling mutations in M_2 seedling populations

Frequencies of dicentric bridges (Fig. 3) and acentric fragments (Fig. 4) increased with soaking time in all radiation treatments. This increase was quite striking in seeds soaked for 6 or more hours in either

nitrogen or oxygen. Even a shorter period of soaking (3 hours) appeared to enhance the X-ray induced chromosomal damage in shoot tip cells (Fig. 4). Soakings in the presence of oxygen produced more X-ray induced aberrations (Fig. 4) and dicentric bridges (Fig. 3) than soaking in nitrogen. Soaking alone produced an apparent stickiness of anaphase chromosomes but no fragmentation. Seeds soaked for twenty hours in presence of nitrogen contained more X-ray induced chromosome aberrations than seeds soaked for less or more time. The same relationship was not observed with regard to the induction of dicentric bridges. Their frequency did not decrease when the soaking period was extended beyond twenty hours (Fig. 3).

X-ray induced chromosome damage could not be evaluated in any of the oxygen soaked seeds where the soaking period was longer than 9 hours. Seed germination was quite pronounced in oxygen soakings of

TABLE 1. *Average number of mutations per M_1 mature plant and spike from X-rayed and non-X-rayed barley seeds, soaked in water bubbled with nitrogen for different periods of time at 20° C and dried back to 15 per cent moisture before X-irradiation.*

(Average of four replications).

Amt. of X-ray (Kr)	Soaking in hours	Total number of			Mut/plant	Mut/spike
		Plants	Spikes	Mutations		
30	0	42	70	2	0.0467	0.0285
	6	64	142	9	0.1406	0.0634
	12	131	280	17	0.2977	0.0607
15	0	288	673	33	0.1145	0.0490
	6	486	1244	98	0.2016	0.0788
	12	733	937	74	0.1009	0.0790
	16	369	819	68	0.1843	0.0830
	20	591	1216	99	0.1675	0.0714
	24	395	831	61	0.1544	0.0734
10	0	368	709	22	0.0597	0.0310
	12	833	1500	88	0.1056	0.0487
	20	729	1608	116	0.1591	0.0721
	24	685	1292	71	0.1036	0.0549
0	0	250	543	2	0.0077	0.0037
	12	717	1753	8	0.0112	0.0036
	20	501	1287	6	0.0119	0.0046
	24	583	1120	5	0.0086	0.0034

longer durations and seeds did not survive the dehydration before and regermination after X-radiation.

Most of the seedling mutations recovered in this study were chlorophyll mutations. In non-irradiated seeds, the number of seedling mutations increased with soaking time from 0.0077 in non-soaked seeds to 0.0112 mutations per plant in 12 hours nitrogen soaked seeds. This difference was significant at the 5 per cent level (Table 1). A similar increase in mutation frequency with soaking time was observed for seeds exposed to 10 Kr. The highest frequency of mutations at this dose was recovered from 20 hour soaked seeds. However, the mutation frequency decreased when the soaking time was increased beyond 20 hours.

The frequency of M_2 seedling mutations from oxygen soaked and irradiated seeds are not recorded here owing to the paucity of data. Low survival, low tillering and high sterility of M_1 plants in these experiments resulted in very low numbers of M_2 seedlings.

IV. DISCUSSION

In the present series of experiments, seeds which had been soaked for different periods of time were always dried back to a constant moisture level (15 per cent) before irradiation. In spite of this partial dehydration, the response of the seeds to X-radiation was the same as that of soaked seeds. Thus, some permanent changes were introduced by soaking which persisted up to and during irradiation. The presence of water *per se* could not have contributed significantly to the increased susceptibility of seeds to radiation damage. On the other hand it seems plausible that substances which offer some protection from X-radiation may have been leached out by the soaking process leaving the sensitive sites more "exposed". These substances *in situ* may partially protect the seeds from radiation damage in any of a number of ways, such as, combining with and thus distracting the possibly mutagenic free radicals produced by X-radiation (cf. GRAY, 1954). The possibility that increased susceptibility to X-radiation by soaking may be due to loss of protective agents from the seed has not been considered in previous work.

Analysis of the oxygen-free, distilled water (pH 5.8) in which the seeds were soaked (leachates) showed many biologically important substances leached from the seeds (KAMRA *et al.*, 1960). The substances were water soluble and apparently occurred free in the seed. Leached substances included many nitrogenous compounds (ninhydrin positive) among which were 14 to 16 amino acids, an amino acid amide, sugars,

sugar phosphates, organic acids, organic ester, many inorganic elements and possibly other unknown compounds. Loss of amino acids could be shown after only 15 minutes of seed soaking, indicating that even a limited exposure to water may change physiochemical characteristics of the seeds. The amount of substances leached increased with time of soaking.

The role of energy-rich compounds like ATP and of protein synthesis in the chromosome rejoining process has been demonstrated by WOLFF (1957, 1958) and KIHLMAN *et al.* (1957). Sugars, sugar phosphates and amino acids which exist free in the seeds and are readily available as sources of energy and many inorganic elements may be very important in the repair of radiation damage, and may even protect seeds to some extent by acting as absorbers of radiation energy.

It is apparent that with increasing times of soaking the seeds become more radiosensitive and lose increasing quantities of chemical substances. Although direct relationship between the two phenomena has not been proved, the loss of energy-rich substances from seeds during soaking may be responsible to a considerable extent for the increase in X-ray damage observed.

It is possible that some automutagenic agent might be produced in soaked seeds which, in turn, may alter the sensitivity of seeds to X-radiation. This assumption gets some support in an indirect way from the extensive literature on this subject (cf. D'AMATO and HOFFMAN-OSTENHOF, 1956) and by the recent work of RIEGER and MICHAELIS (1958 a and b). It is interesting to note that MICHAELIS *et al.* (1959) suspect this automutagenic substance to be ethyl alcohol in *Vicia faba* whereas LOVELESS and REVELL (1949) showed a negative effect of ethyl alcohol as a mutagenic agent in *Vicia faba* seeds.

That some important changes in the sensitive sites during soaking had taken place is demonstrated by the mutation studies. It is significant to note that an increase in seedling mutation frequency was observed in non-X-rayed, soaked seeds. This phenomenon was not accompanied by any apparent damage to the M_1 seedling growth or the mitotic chromosomes in the M_1 shoot tip cells.

It has been reported that water soaking of seeds causes an increase in the frequency of spontaneous chromosome aberrations in *Vicia faba* roots (LEVAN and LOTFY, 1950; RIEGER and MICHAELIS, 1958, a and b; MICHAELIS and RIEGER, 1958). Recently it has also been reported (MERZ, 1958) that extended anaerobiosis to *Vicia faba* roots will cause chromosome breaks. In barley, on the other hand, no such increase in chromo-

some aberrations was observed by RIEGER and MICHAELIS (1958 a). An increase in the stickiness of mitotic anaphase chromosomes was the only damage observed. This is in complete agreement with the findings reported here and points out an interesting difference between barley and *Vicia faba*.

Oxygen-soaked seeds were damaged more by X-rays than nitrogen soaked seeds. This greater sensitivity was possibly related to the meristematic activity in the seeds since those soaked for more than 9 hours in oxygen were initiating roots and shoots.

A quantitative relationship between time of soaking and amount of X-ray damage was evident, *i. e.*, within any given radiation treatment the seedling height (Figs. 1 and 2) decreased with increased soaking. This effect usually reached a maximum at 20 hours, following which there was an apparent recovery with increased soaking. A somewhat similar effect was observed earlier for wheat by HENSHAW and FRANCIS (1935), for barley by WERTZ (1940, 1941) and more recently by BIEBL (1959) in wheat.

Any explanation of the somewhat decreased X-radiation susceptibility of the seeds when the period of soaking was extended beyond 20 hours can only be speculative. The "recovery" may be entirely self-acquired, by regeneration of the lost substances by degradative and synthetic enzyme systems in the seed which become activated during soaking.

Seeds soaked at 30° C were damaged more by X-rays than those soaked at 20° C (Figs. 1 and 2). In oxygen-soaked seeds this may be due to more advanced development at the higher temperature. Greater radiation damage to nitrogen-soaked seeds was also associated with higher soaking temperature even though no sign of germination was apparent. It is well known that increased temperature accelerates chemical reactions. The permeability of the seed coat may also be affected.

Chromosome damage in shoot tip cells of seeds and growth of seedlings were affected to the same degree by the various treatments. X-ray induced chromosome aberrations increased and seedling height decreased with soaking time. These were greater in oxygen-soaked than in nitrogen-soaked seeds. Both criteria showed reduced damage when soaking time was extended beyond 20 hours. However, it is not possible to say if this reduction in damage is due to increased chromosome rejoining or to a lower induction of chromosome breaks.

Mutation frequencies were similarly affected by the treatments. X-ray (10 Kr) induced mutation frequency in M_2 seedling populations increased with increasing time of soaking and was greatest in 20 hour

soaked seeds. Seeds exposed to higher X-ray doses (15 and 30 Kr) led to a decline in mutation frequency which may be due to intrasomatic (EHRENBERG, 1953) or diplontic (GAUL, 1957) selection. As pointed out earlier non-X-rayed but soaked seeds yielded significantly higher frequency (5 % level) of seedling mutations than non-soaked seeds. Since soaking alone did not produce visible chromosome breaks but only anaphase stickiness, these mutations are most likely point mutations. A change in the chemical environment brought about by an altered cell physiology as shown by the stickiness of mitotic chromosomes, and loss of many organic and inorganic substances (KAMRA *et al.*, 1960) may be the cause of mutations.

SUMMARY

The influence of soaking on radiation induced damage to barley seeds was investigated. Seeds were soaked in water at different temperatures and in the presence of either nitrogen or oxygen and were dried back to 15 % moisture so that they were essentially "resting" at the time of X-radiation. The damage due to X-radiation was determined by measuring (1) seedling height of M_1 seeds; (2) chromosome aberrations in shoot tip cells of M_1 seeds and (3) seedling mutations in M_2 seedling populations. Changes in radiation response of soaked seeds were compared with changes in the kind and amount of substances leached (KAMRA *et al.*, 1960).

X-ray induced damage to M_1 seedling growth, chromosome aberrations in shoot tip cells of M_1 seeds and M_2 seedling mutations increased with increasing time of seed soaking before X-radiation. In addition, the damage increased with the presence of oxygen during soaking and with higher temperature of soaking. Seeds soaked for 20 hours were usually most susceptible to X-rays; additional soaking led to some reduction of the X-ray induced seedling damage. As little as 3 hours of seed soaking changed the X-ray induced breakage observed in mitotic chromosomes but this was not obvious from seedling height measurements. Soaking by itself did not produce breaks but increased stickiness.

A significantly higher mutation frequency was observed for non-irradiated but soaked seeds than for non-soaked seeds.

Soaking and drying back to constant moisture level of 15 % appeared to cause a permanent change in the seeds which persisted up to and during X-radiation. Thus, the increased X-ray sensitivity of soaked

seeds is not entirely due to indirect effects of water, but in part may be related to the nature and amounts of substances lost by leaching (KAMRA *et al.*, 1960).

Seed soaking alone appears to modify the physiochemical properties of chromosomal material as indicated by the stickiness of mitotic chromosomes. This in turn, may be due to an alteration in the chemical environment of the cells brought about by the loss of many energy-rich compounds in the process of soaking.

It is apparent that with increasing times of soaking the seeds become more radiosensitive and lose increasing quantities of chemical substances. Although direct relationship between the two phenomena has not been proved, the loss of energy-rich substances from seeds during soaking may be responsible to a considerable extent for the increase in X-ray damage observed.

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AN INVERSION IN BARLEY

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IN the literature, only few cases of inversions in barley are known (SMITH, 1941; BHADURI and SHARMA, 1949; DAS, 1955). Anaphase I bridges with fragments are seldom found in barley, untreated or X-irradiated, presumably because chiasmata are mostly terminal, hence only inversions including the chromosome ends will form bridges and fragments. Recently, HAGBERG (unpubl.) found two cases in barley with a bridge and a fragment in low frequency, indicating a duplication or an inversion of a proximal chromosome segment.

The present inversion was detected in 1956 in a population (strain No. 01518) appearing after X-irradiation of the two-rowed barley variety Ymer (GUSTAFSSON and NYBOM, 1949). It is true that a duplication, large enough to allow chiasma formation, might result in a bridge and a fragment at anaphase I, but this possibility is less probable in the present case, since no appreciable change in size of any of the chromosomes was seen. Cytogenetic analysis showed the inversion to be associated with:

(1) a factor for microspore (tetrad unit) necrosis;
(2) a recessive sublethal *xantha* gene; and
(3) a factor for male sterility (partly multi-ovarial).

As far as seed fertility is concerned, the inversion heterozygotes, cytologically identified, can be separated into four groups (I—IV, Table 1). All of these groups show bridge and fragment formation at anaphase I, although in different frequencies, viz. 46 per cent in group I and 29 per cent in II, while the frequencies of other similar aberrations, one bridge and two fragments, a double bridge and two fragments etc., were 3 and 20 per cent, respectively, for the two groups. The theoretical seed fertilities, calculated under the assumption that meiotic aberrations result in lethality to the gametes affected, are 74 per cent for group I and 66 per cent for group II. Only the former value deviates significantly from the value observed. This deviation may possibly be due to pre-

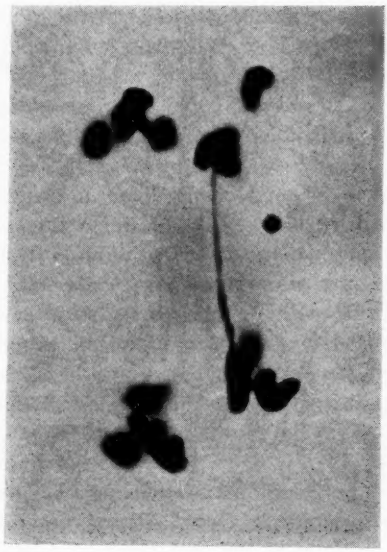

Fig. 1. A bridge and a fragment at first anaphase in an inversion heterozygote.

ferential segregation of the macrospores. The aberration frequencies of groups III and IV have not yet been determined.

The percentages of "good pollen" in group I, II and IV differed only slightly from the corresponding seed fertilities. The pollen fertility of group III, however, was significantly higher than the seed fertility, which is an unusual situation in barley.

The microspore (tetrad unit) necrosis (1, above) was found only in group IV, and is thus probably not caused by the inversion. The necrotic microspores were of the same size as the normal ones, but their

TABLE 1. *Seed fertility, pollen fertility and meiotic irregularities.*

Group no.		I	II	III	IV	Bonus barley
Fertility %	Seed	84	69	50	26	96
	Pollen	84	72	69	26	95
% PMC: s	One bridge and fragment	46	29	—	—	0
	Other aberrations	3	20	—	—	0
	No aberrations	51	51	—	—	100

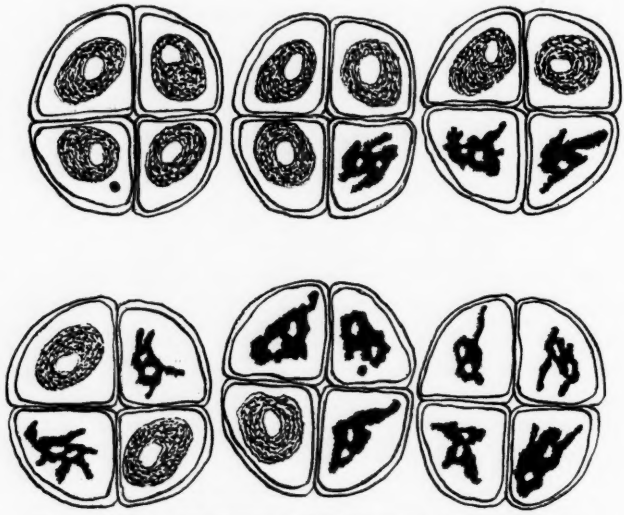

Fig. 2. The six tetrad types of fertility group IV (see Table 2).

nuclei were irregular in shape, and pycnotic. They often contained two nucleoli instead of the one normally found (Fig. 2). In each tetrad, necrosis could affect 0 to all four microspores. In the case of two microspores being affected, they could be oriented in two ways, side by side or diagonally. The frequencies of the six different tetrad types, thus distinguished, are recorded for 360 tetrads in Table 2.

It is seen that the differences between observed and expected show a marked excess of the tetrad types 1 and 6, that is the entire tetrad either

TABLE 2. *Incidence of necrosis within the tetrads.*

Type no.	Necrotic cells per tetrad	Frequency %			Difference observed—exp.	
		Observed		Expected	Present case	*Petunia*
1	0	10.0		2.1	+7.9	+6.9
2	1	10.3		13.6	−3.3	−1.3
3	2 (side by side)	17.2	26.1	33.3	−7.2	−7.5
4	2 (diagonally located)	8.9				
5	3	27.2		36.2	−9.0	−8.8
6	4	26.4		14.8	+11.6	+10.7

alive or dead. It is of interest that these values show a striking agreement with a similar material studied by LEVAN (1942) in a *Petunia* strain, in which the pollen grains remained permanently in tetrads (Table 2, last column). LEVAN's interpretation of this disagreement between observed and expected was that the dying of one pollen grain in a tetrad enhanced the dying of more pollen grains in the same tetrad. Thus tetrad types 2—5 of Table 2 would represent stages in an accelerated process, the end result of which will be the accumulation of tetrads into type 6. It is doubtful whether this hypothesis may account for the present data, since conditions were somewhat different. In *Petunia* observations were made on ripe pollen, in which the dead pollen grains were empty and often smaller than the living pollen grains, especially those of tetrad type 1, while in the present case much earlier stages were examined, no size difference being found either between living microspores of tetrad type 1 and the other types, or between living and dead microspores.

Since no data on the frequency of the inversion bridge are available so far for this group, nothing is known about possible correlations between the necrosis and the inversion. The inversion fragment, however, was still detectable as a micronucleus in the cytoplasm of the microspores and even of the ripe pollen grains. Normal microspores were found to have a lower proportion of cells with one, or seldom two, micronuclei than necrotic microspores. The following frequencies were recorded:

	No micronucleus	One or two micronuclei
Normal microspores	85	8
Necrotic microspores	120	31

$\chi^2 = 5.2$ $\quad n = 244$

As, presumably, the inversion fragment is distributed at random to the tetrad units, this deviation, which is significant on the 5 per cent level, indicates a causal connection between the fragment and the necrosis.

The fact that an odd number of microspores in a tetrad may be necrotic makes it difficult to find a simple genetic interpretation of the necrosis. Furthermore, the first and second cleavage planes cannot be distinguished in the tetrads. Consequently, it could not be decided whether two necrotic cells in a tetrad were sister cells within one dyad, or belonged to different dyads.

Since plants of group IV only give progeny plants with similar fertility, it may be concluded that the homozygotes and about half of the

heterozygotes are eliminated, this actually constituting a system of balanced lethality. Three other similar cases have been found in my barley material, one after X-irradiation, the other two after myleran treatment.

When the *xantha* gene (2, above) occurs in plants of fertility group IV, the segregation ratio of green plants to *xantha* plants is about 1 to 3. The green plants are all heterozygous for the *xantha* gene. This indicates some degree of linkage between the normal allele of the *xantha* locus and the inversion in this fertility group.

The male sterile plants (3, above) were characterized by a partial transformation of the anthers into ovaries, similar to what is reported recently by KAMRA and NILAN (1959). Judging from the meiosis of these plants, they were not inversion heterozygotes. All plants showed "cytomixis" during pachytene, and in some of them a chromatic body, resembling a heteromorphic bivalent was observed at metaphase I. The pollen grains were of normal appearance, but considerably smaller than in normal plants. The anthers that were not transformed into ovaries were hard and did not dehisce. Attempts to fertilize with such pollen were unsuccessful.

Further genetical and cytological investigations on this material are in progress.

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THE MUTAGENIC EFFECT OF 8-ETHOXYCAFFEIN, CAFFEIN AND DIMETHYLSULFATE IN THE OPHIOSTOMA BACK-MUTATION TEST

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I. A STUDY OF OPHIOSTOMA AS A TEST OBJECT IN THE BACK-MUTATION TEST

1. Introduction

OPHIOSTOMA MULTIANNULATUM HEDGC. et DAVIDS is an ascomycete living on insufficiently dried pine wood. When it is grown in pure culture two morphological forms can be distinguished: in liquid medium with good aeration conidia are produced propagating themselves by budding whereas on agar medium distinct mycelia are formed. These ways of growing make it suitable for a back-mutation test. As a great number of biochemical mutants have been induced by FRIES (1948), a considerable experimental material was available.

2. Methods

In a previous report (ZETTERBERG and FRIES, 1958) a brief description was given of the methods and results of the screening for spontaneous back-mutations in biochemical mutant strains of *Ophiostoma*. Spontaneous back-mutations to prototrophy were quite a common phenomenon among strains whose mutant character had been induced by irradiation with ultraviolet light, whereas such reversions were less demonstrable in strains where the auxotrophy had been caused by x-rays.

Among nearly 100 strains tested eight were selected for further experiments. These strains were thought to satisfy the requirements for a

suitable test object in the back-mutation test. The requirements were as follows:

1. Spontaneously occurring prototrophs must not be too frequent. This is further discussed on pp. 285—287.

2. The conidia which have reverted to the wild type should grow out to distinct mycelia on the minimal agar plates used, thus facilitating the counts.

3. The conidia which have not mutated back to the wild type must not grow to any great extent as they would make the plates translucent and confuse the counting of the wild-type mycelia. Since the plates are heavily loaded with conidia (about 5×10^7), even a moderate outgrowth of all conidia might result in the exhaustion of the nutrients in the medium (RYAN, 1953). On the other hand, only zero-point mutations would be detected if the mutant conidia entirely failed to grow. In the strains used in this investigation a slight growing out of most living conidia was observed on minimal agar. Suppression of wild-type conidia, caused by this growth (cf. GRIGG, 1952), was in no case observed. The strains were especially tested in this respect. However, it was noticed that if almost all conidia are killed (not applicable to these experiments), all the survivors will form small mycelia even on minimal agar, the latter obviously enriched with the necessary metabolite by diffusion from autolysed cells.

4. The growth should be exclusively conidial in liquid medium. Occasional hyphal fragments can, however, be filtered away by means of sterile cotton. The conidia are usually uninucleate (VON HOFSTEN, 1959), but since no synchronization of cell division was tried, and since small hyphal fragments may occur, a very slight part of the cell population may be at least binucleate.

The methods are generally the same as those used in *Neurospora* back-mutation test (GILES and LEDERBERG, 1948; KÖLMARK and WESTERGAARD, 1949).

Mutation frequencies are expressed as the total number of reversions, per 10^6 living conidia, occurring during the course of the experiment and giving observable mycelia during the incubation time. The length of the latter depends upon the growth rate of the strain (viz., the growth rate of reversions on minimal agar), but is always more than 4 days, thus allowing all delayed mutations to be phenotypically expressed. The incubation time has seldom exceeded 7 days.

The conidia were grown at 30° C in Pyrex flasks on a shaker. The medium was liquid "modified medium 3" (FRIES, 1949), to which was

added the metabolite specifically required by the mutant strain. When the cultures were in the logarithmic phase of growth, the conidia were centrifuged down and washed twice in sterile distilled water. The mutagenic treatment was then applied. Simultaneous control experiments under identical conditions, except for the mutagenic treatment, were performed in each case with material from the same conidial suspension.

The frequency of wild-type cells of spontaneous origin was obtained from the control experiments. 10 plates (A) were prepared by mixing 15 ml of the minimal medium ("modified medium 3" solidified with 1.5 per cent agar) with 1 ml of the conidial suspension, the density of which had been determined by counts in a haemocytometer and was usually of the order of 5×10^7 conidia per ml. On these plates, only those conidia grew which had reverted to the wild type. The mycelia, formed by them, could be easily counted. As a test for the Grigg-effect, 10 plates were incubated with a suspension diluted 20 times (2.5×10^6 conidia per ml). This test was omitted after it had been proved that the method could not be criticized in this respect, when using these strains. However, in cases where a high frequency of wild types was expected, the test was included.

The initial viability of the conidia was tested by inoculating 10 plates (B) of minimal medium plus the metabolite specifically required by the mutant strain with 1 ml suspension diluted to contain about 200 conidia per ml. On these plates all living conidia grew.

Those conidia which had been treated with the expected mutagen were tested in an analogous way. On 10 plates of type A prototrophic mycelia developed, emerging partly from conidia which had reverted spontaneously, partly from conidia which had reverted on account of the mutagenic treatment. The viability test on plates of type B gave the number of conidia surviving the treatment.

In the viability tests in those experiments where double mutants had been used, it was necessary to add to the plates both of the metabolites specifically required by the strain. Only one of the metabolites was added when an investigation was made into the frequency of cells with a lost requirement for the other metabolite.

Thus, the experiments gave the following information: 1. The percentage of living conidia before the treatment. 2. The percentage of surviving conidia. 3. The frequency of wild-type cells of spontaneous origin per initially living conidia. 4. The induced back-mutation frequency per initially living conidia. 5. The frequency of back-mutations per surviving conidia.

3. The reliability of the Ophiostoma back-mutation test

In spite of every effort to obtain identical conditions from one biological experiment to another, the results cannot be expected to be identical, even if it is possible to work, as in the experiments described here, with material which is homogenous genetically. According to DEMEREC et al. (1951) every change in metabolism may cause a mutation. KÖLMARK and WESTERGAARD (1949), by using the *Neurospora* back-mutation test, have shown that the viability can vary considerably after treatment with the same mutagen under identical external conditions.

Even if we disregard the difficulty of overcoming the biological variation which manifests itself principally as a variation between identically constructed experiments on different occasions, the use of back-mutation tests also involves other variation possibilities, which can influence the conclusions of an experiment.

Obviously, the cells used in an experiment must belong to the same population. For technical reasons it was not possible to grow the conidia in one flask only, and therefore the conidia were mixed during the washing procedure. One part of them was then used in the control experiment and the other in the induction experiment. If the latter treatment involved the suspension of the conidia in a solution containing a chemical mutagen, the cells had to be washed a further two times to get rid of the substance. The densities of the untreated control and the treated cell suspensions had therefore to be calculated independently. The process of calculating the suspension densities in the haemacytometer thus involves a variation possibility. Four different samples were taken from each suspension, and the density was determined by the values obtained in counting 4×5 squares. The standard error for such a calculation was determined in 20 cases, using the formula

$$\frac{S(x-\bar{x})^2}{n(n-1)}.$$

The mean value turned out to be ± 3.46 per cent. This uncertainty can be ignored, when irradiation with ultraviolet light was used as the mutagen, because the suspensions of the control and the induction experiment were taken from the same population, and no processes were performed where the density could be modified.

The procedure of diluting the suspensions for the viability test also provides a moment of uncertainty. The result of the viability test influences the calculation of back-mutation frequency, which is expressed

per 10^6 living conidia (see Table 1). A model experiment was constructed to test the reliability of the dilution and plating procedures. A suspension containing 10^9 conidia/ml was diluted to contain 100 conidia/ml (100 times in each of three dilutions). The values obtained from this experiment showed that the whole process (dilution and plating) had a standard error of 5.8 per cent.

Undiluted suspensions were inoculated on the plates containing minimal agar; the absolute number of reversions could therefore not be influenced by dilution errors. The last process in the model experiment described above was the plating, and the mean standard error in 10 series of plates was 3.31 per cent. By this means it was concluded that the determination of the absolute number of reversions was subject to similar deviations. All dilutions and platings were prepared by the author.

As stated above, the mutation frequencies are expressed as the number of reversions per 10^6 living conidia, calculated by the formula

$$\frac{10n}{d \cdot v},$$

where d equals the density of suspension (the number of conidia per $ml \times 10^{-7}$); v equals the viability in per cent; n equals the number of reversions per ml suspension.

If it is assumed that the factors vary within their parameters by 2 σ (double the standard errors), it is possible to establish a formula for the maximal deviation

$$\frac{10(n \pm 2\sigma_n)}{(d \pm 2\sigma_d)(v \pm 2\sigma_v)} - \frac{10n}{d \cdot v}.$$

It is evident that the maximal positive error is obtained when σ_n is given a positive sign, while σ_d and σ_v have negative signs. The maximal negative error is obtained when the parameters are given the opposite signs.

With the assumptions above, the maximal errors can be expressed as a percentage of the calculated value by the formulas

$$100\left[\frac{dv(n+2\sigma_n)}{n(d-2\sigma_d)(v-2\sigma_v)} - 1\right] \text{ (positive error), and}$$

$$100\left[\frac{dv(n-2\sigma_n)}{n(d+2\sigma_d)(v+2\sigma_v)} - 1\right] \text{ (negative error),}$$

which are constant for all real values of d, v and n, when the parameters are constant.

TABLE 1. *The number of wild-type cells (per 10^8 living conidia) in different control experiments.*

Strain 2494	Strain 2505	Strain 2614	Strain 2615	Strain 2626	Strain 2657	Strain 2511	Strain 2668	Double mutant uracil-hypox.-less		Double mutant uracil-meth.-less	
								$\bar{u}_{2505}$	$\bar{h}_{2494}$	$\bar{u}_{2505}$	$\bar{m}_{2511}$
2.5	0.02	0.51	< 0.05	0.60	4.7	< 0.01	0.08	0.65	0.06	0.13	0.02
0.22	1000	0.1	0.10	650	104	0.18	0.08	0.37	< 0.02	0.13	0.04
0.7	33.8	1.4	2.9	4.8	∞	0.03	0.10	0.21	< 0.03	0.5	∞
∞	1.6	< 0.01	3300	∞	∞	0.15	∞	0.67	0.47	1.5	∞
∞	1.0	< 0.01	0.14	∞	∞	∞	0.03	< 0.1	< 0.1	1.5	∞
∞	1000	< 0.01	0.14	∞	∞	∞	0.02	0.11	0.06	316	0.67
0.038	4500	0.007	0.10	∞	450	0.014	0.01	0.05	0.20	0.80	0.10
2.3	4500	< 0.01	0.04	∞	∞	0.26	0.01			1.26	0.03
0.28	2200		0.09	∞	50		0.05			0.62	0.096
0.28	3.1		0.025	0.40	50		0.11			1.1	∞
230	0.3		0.012	0.40	39					0.79	0.03
0.1	0.64		0.22	0.41						0.33	∞
0.11	0.28		0.03	9.6							
∞	0.31			0.88							
1.65	1.4			0.6							
0.51	1.0			0.30							
0.36	86										
∞	0.90										
0.09	0.18										
0.014	0.38										
∞	0.32										
0.11	0.49										
0.15											
0.06											
0.04											

37

In these experiments, where the values of the standard errors are $\sigma_d = \pm 3.46$ per cent; $\sigma_v = \pm 5.8$ per cent; and $\sigma_n = \pm 3.31$ per cent, the maximal positive error was calculated to be 29.8 per cent and the negative error 21.7 per cent.

When the result of an experiment seemed doubtful, the experiment was repeated. In some cases other clarifying experiments were performed.

4. The significance of different frequencies of wild-type cells in the mutant strains

Table 1 shows that the number of wild-type cells per million living cells in the mutant strains varied considerably. It is most improbable that this was caused by such enormous variations in the spontaneous back-mutation frequencies occurring from one experiment to another. The phenomenon might be of the nature of population dynamics and might imply that prototrophic conidia of back-mutation origin were selectively superior to the auxotrophs. During growth, the wild-type cells would thus make up a greater part of the population than could be accounted for by back-mutation. According to ATWOOD, SCHNEIDER and RYAN (1951) the selection would be of critical importance in a continuously growing culture of stored-culture type, where selectively superior types would be more likely to appear among the auxotrophs, which constitute by far the larger number. This superiority in numbers would remain, and although the frequency of prototrophs would fluctuate, it would seldom reach other than very low values. This explains why stored cultures are nearly constant over long periods of time. This interpretation can probably also be applied to *Ophiostoma*.

The experiments with strain 2494 (Table 1) showed that the frequency of wild-type cells fluctuated widely. The highest figures could not be estimated (represented by ∞ in Table 1). It can be seen that the high values are most frequent in the earlier experiments, when the following method of inoculation was used. Bits of mycelia were taken directly from the stored cultures and transferred to liquid medium. These mycelia might have contained many wild-type cells which multiplied during the growth in liquid medium, and, at the time when the conidia were treated and spread on agar plates, the wild types would sometimes constitute up to a few per cent.

If the first inoculum can be genetically well defined, only one cell, demonstrably auxotrophic, should be used. This is technically impos-

sible. The next best procedure would be to use as inoculum a small uniconidial mycelium, i.e., a mycelium directly developed from a single conidium. Then the growth would originate from comparatively few cells of a definable phenotype. The wild types, caused by reversions during growth, reach a number that corresponds better to the mutation frequency, the fewer the number of cell generations. Owing to the rarity of reversions there is little chance that they will occur at the beginning of growth. This method was found to be rather reliable, and the percentage of prototrophs was generally lower and more constant in the later experiments. This fact indicates that selection is common in favour of the wild type in the stored agar cultures, but the effect of the same selection, if it exists, during growth in liquid medium is unimportant.

Strain 2657 is an exception in this respect. Here the portion of prototrophs was always very high in comparison with the other strains. This might depend on a strong selection favouring the prototrophs, or on a very high spontaneous back-mutation frequency, or on a combination of these two factors.

Strain 2657 is evidently unsuitable for the back-mutation test on account of the high frequency of wild-type cells. The reason for describing the experiments with this strain is that they demonstrate how a high background of wild-type cells masks the action of the mutagens. If the method used were 100 per cent correct, it would be possible to establish the absolute number of induced back-mutations by subtracting those counted in the control experiment from those obtained in the induction experiment with a correction for the lower viability caused by the mutagenic treatment. In most cases the number of induced prototrophs of strain 2657 would be low in comparison with the number already present in the population. The result of the viability test, therefore, plays a very important role. For instance, the experiment with dimethylsulphate in Table 5: 16 would have shown a quite different result if the viable conidia had amounted to 10 per cent instead of 6 per cent, which was the number calculated. The number of back-mutations per 10^6 surviving conidia would then have been 24 instead of 70, i.e., a reduction instead of an expected increase.

Some authors use the system of defining the mutagenic effect as the number of times the induced mutation frequency exceeds the spontaneous one. The present author prefers to express the result of a mutagenic action as the number of back-mutations after treatment minus the number of spontaneous back-mutations as registered in the control experi-

ment. It is supposed that the spontaneous frequency means nothing other than a kind of contamination by wild-type cells due to technical insufficiency of the method. The shortcomings of the first system of notation are clearly demonstrated as follows: Suppose that in two identical experiments with the same strain, the frequencies of the prototrophs in the control experiments were 100 and 0.01 per 10^6 viable conidia, and that after UV-treatment the frequencies were 110 and 10 per 10^6 survivors, respectively. With the first system of notation the increase caused by UV would be 1.1 times and 1000 times, respectively. If the background approaches zero, this increase would approach infinity. If the latter method of notation is used, the result will be that about 10 back-mutations per million survivors will be induced in both experiments. The first experiment would in any case be less reliable as discussed above.

One must entertain grave doubts about the possibility of critically determining the spontaneous mutation frequency by experiments of the above type. The fact that most experiments give numbers that are low and tend to lie around a probable average value must not conceal the possibility that a certain selection in one direction or the other may have a considerable influence. However, if the external conditions are the same under a series of experiments, it may also be expected that both mutation frequency and strength of selection are the same. The number of wild-type cells obtained is the result of the action of these factors. Very extensive experiments are probably required to decide on the real spontaneous mutation frequency in a population of cells which is several generations old.

5. Double mutants

A comparison between the mutabilities of two different genes is most reliable if the two genes can be placed in the same genetic and physiological environment, i.e., in the same genome. According to GLOVER (1956), differences in the genetic background may influence the mutability. DEMEREC et al. (1951) claim that any metabolic disturbance can cause a mutation, and KÖLMARK (1953) points out that differences in physiology and permeability may influence comparisons between genes in different environments.

Two double mutants with different physiological requirements were made, one by crossing strain 2505 with strain 2494 and the other by crossing strain 2505 with strain 2511. In the first case a uracil-hypoxanthine-less double mutant was obtained. The outcrossing of 10 wild-type:

3 hypoxanthine-less: 9 uracil-less: 10 uracil-hypoxanthine-less spores indicates that the two genes in question are not linked. The other crossing gave an uracil-methionine-less double mutant. The ratio 4 wild type: 5 methionine-less: 3 uracil-less: 4 uracil-methionine-less also indicated an independent assortment.

When the mutant strains were incrossed with wild-type (+) or (—) mycelia in order to obtain or improve the ability to form perithecia when crossed with other mutants, the general genetic constitution might be changed. However, the specific mutant character could be detected in the offspring. These facts should be kept in mind when considering the influence of the genetic background on the mutability of a gene occurring in a single mutant strain as well as in a double mutant together with another gene causing a double extra requirement. In the latter case the genetic background may be changed not only by the introduction of a new mutant allele. It is also possible that the cross may cause a far greater change in the genetic constitution, the testing of which is not yet possible.

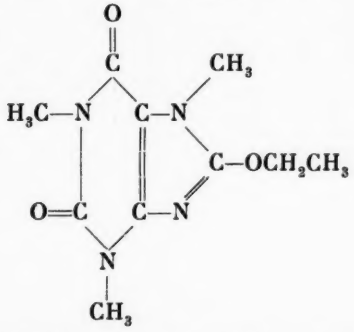
II. THE MUTAGENIC EFFECT OF FOUR TREATMENTS INVESTIGATED BY MEANS OF THE BACK-MUTATION TEST

1. Introduction

The purpose of this work was to investigate the mutagenic effect on *Ophiostoma* of some purine derivatives, some of which are known for their mutagenic effect on chromosomes in root-tips of *Vicia* and *Allium* (KIHLMAN, 1951, 1952), and some for their mutagenic effect on micro-organisms (FRIES and KIHLMAN, 1948; DEMEREC, BERTANI and FLINT, 1951; NOVICK, 1956). The work was initiated with an investigation of 8-ethoxycaffein, which is known, among the purines, as being one of the most vigorous chromosome breakers. However, since the mutagenic effect of 8-ethoxycaffein was found to be rather weak in *Ophiostoma* under the experimental conditions used, and since caffein was completely without mutagenic effect, it was thought that an extension of the investigation also to include other purine derivatives would give only limited information. For this reason the study of the purine derivatives was not further extended. For comparison, the genetic effect of two well-known mutagenes was then investigated, viz., irradiation with ultraviolet light and treatment with dimethylsulfate.

2. Experiments with 8-ethoxycaffein (EOC)

a. *Methods*



EOC

Chemical properties: Melting point 140° C (FISCHER, 1882). Solubility in water at 25° C 0.3 g per l. (HUSTON and ALLEN, 1934). EOC was kindly supplied by Dr. B. KIHLMAN.

The conidia were treated with a 5×10^{-3} M solution of EOC in distilled water. Owing to the low solubility of EOC the solution was boiled up and immediately chilled to 15° C to give a supersaturated solution. (According to KIHLMAN, 1952, purines are only slightly toxic, and this toxicity is diminished at lower temperatures. KIHLMAN, 1952, also showed that the radiomimetic effect of EOC is greatest at 12° C and decreases at both lower and higher temperatures.) The conidia were suspended in the EOC-solution, the density of the suspension varying between 2×10^7 and 6×10^7 conidia per ml. Two Pyrex-flasks, each containing 15 ml of suspension, were placed on a shaker at 15° C. The shaking maintained a high oxygen concentration in the suspension and facilitated the respiration of the cells. The radiomimetic effect of EOC is strongly dependent on the concentration of oxygen (KIHLMAN, 1955b). The influence of the duration of the treatment was investigated in all strains used as shown by the examples in Fig. 1. The treatment time was then fixed at 36 hours in the subsequent experiments. In those cases where EOC increases the spontaneous mutation frequency, the increase cannot be detected until 15—20 hours have passed. With some strains the treatment could not be extended much over 36 hours because the number of survivors was too low. Even in the control experiment, where the conidia were suspended in distilled water, the viability decreased too much with prolonged treatments. No growth occurred during the treatment, which was shown by calculating the optical density

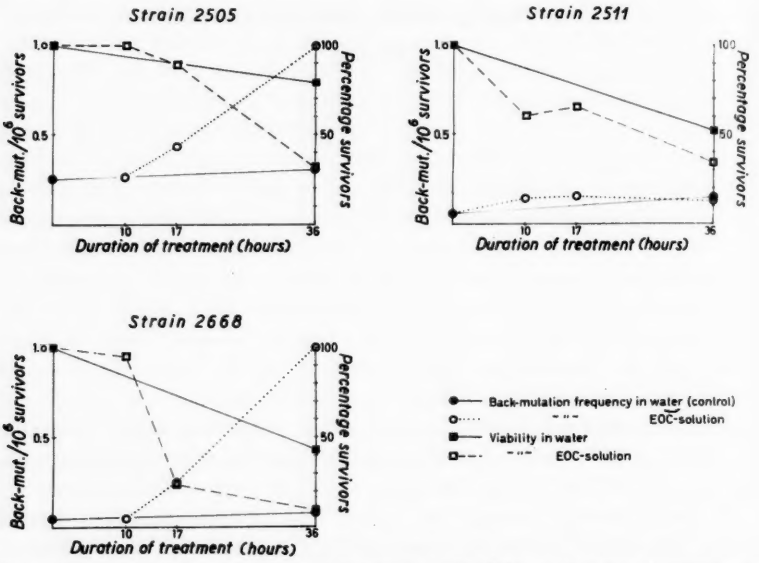


Fig. 1. Influence of the duration of treatment with EOC and water (control) on back-mutation frequency and viability in three biochemical mutant strains of *Ophiostoma*.

of the suspension and by direct counts in a haemacytometer before and after the experiment. The EOC-suspension was removed by centrifugation, and the conidia were washed twice and resuspended in sterile distilled water.

b. *Results and discussion*

The results of the experiments with EOC are summarized in Fig. 1 and Table 2. It is evident that the current influence of EOC on the back-mutation frequency is slight in comparison with the effect after UV-irradiation (Table 6).

WESTERGAARD et al. (1951) considered a treatment mutagenic if, by using the *Neurospora* back-mutation test, it resulted in a 5 times' absolute increase in the frequency of wild-type cells. If that criterion were applied to these experiments also, the conclusion would be that EOC has no mutagenic action. However, in the opinion of the present author a treatment with any agent resulting in an increase of the spontaneous mutation frequency should be considered mutagenic, if there can be

TABLE 2. *Results of treatment with EOC.*

Exp. no.	Strain	Investigated reversion	Control		Treated series			
			Back-mut. per 10^6 living conidia.	Total no. of back-mutations.	Back-mut. per 10^6		Per cent surviving conidia	Total no. of back-mutations.
					Initial conidia	Surviving conidia		
1	Uracil-less 2505		0.31	66	0.38	0.99	39	79
2			0.18	110	0.18	1.2	15	89
3			0.30	100	0.18	1.8	10	41
4			0.38	258	0.43	0.54	79	270
5		u^-_{2505}	0.50	120	0.80	2.4	32	180
6	Uracil-hypox.-less	to	0.21	46	0.13	1.3	10	28
7	double mutant.	u^+_{2505}	0.11	20	0.05	0.24	21	7
8			0.06	4	0.035	0.07	50	2
9	Uracil-meth.-less		0.13	69	0.17	0.45	39	61
10	double mutant.		316	2350	228	253	90	1586
11			0.80	215	0.62	1.4	44	135
12			1.26	324	1.5	3.1	48	324
13	Methionine-less 2511		0.15	65	0.09	0.13	66	28
14		m^-_{2511}	0.26	78	0.10	0.37	27	52
15	Uracil-meth.-less	to	0.005	3	0.02	0.04	39	6
16	double mutant.	m^+_{2511}	0.67	5	1.4	1.4	100	9
17			0.10	27	0.09	0.21	44	20
18			0.03	9	0.05	0.10	48	13
19	Hypoxanthine-less	h^-_{2494}	1.7	612	0.89	2.2	41	235
20	2494	to	0.36	79	0.34	0.34	100	45
21	Uracil-hypox.-less	h^+_{2494}	0.11	7	0.017	0.035	50	1
22	double mutant.		0.06	9	0.01	0.17	21	5
23	Uracil-less 2614	u^-_{2614}	1.4	120	1.4	12.5	11	144
24		to		0			2	1
25		u^+_{2614}		0			23	0
26				1			53	1
27	Uracil-less 2615	u^-_{2615}	2.9	745	1.7	3.5	48	569
28		to u^+_{2615}	0.10	28	0.08	0.16	41	22
29	Uracil-less 2626	u^-_{2626}	4.8	253	1.6	4.3	37	49
30			0.41	96	0.35	1.3	27	81
31		to	9.6	755	7.4	9.2	80	550
32		u^+_{2626}	0.88	136	0.92	0.92	100	159
33			0.30	40	0.17	0.20	77	15
34	Uracil-less 2657	u^-_{2657}	104	827	47	117	40	339
35		to u^+_{2657}	39	4420	17	54	31	1890
36	Methionine-less	m^-_{2668}	0.08	10	0.22	1.0	22	26
37	2668	to	0.05	6	0.15	1.0	15	16
38		m^+_{2668}	0.11	8	0.36	0.41	90	19
39			0.03	3	0.04	0.2	20	4

established with acceptable certainty that an increase has actually occurred.

From a theoretical point of view it would be very interesting to find a reliable answer to the question of whether or not EOC can act as a mutagen in experiments of this kind. In the light of the weak effect of EOC and the possible variations due to the method, the experiments were repeated several times, and in some cases the results were clarified with experiments of a different kind.

A significant absolute increase of the wild types was obtained in only 4 experiments (Table 2: exp. 5, 36, 37, 38). The latter three gave strong evidence that EOC is a mutagen for the gene, the wild-type action of which is blocked in the methionine-less strain 2668. The other gene causing methionine deficiency (m^-_{2511}) showed no clearly increased back-mutation frequency by EOC-treatment of strain 2511 (Table 2: 13, 14), whereas the same gene in a uracil-methionine-less double mutant was affected to a detectable degree in every one of the four experiments. The figures in experiments 15 and 16 were calculated on the basis of very few back-mutations, and the increase may be accidental. However, the observed increase of the back-mutation frequency in experiments 17 and 18 was based on better evidence and was considered real. The gene causing hypoxanthine deficiency (h^-_{2404}) did not change its stability by treatment with EOC. The reversion rate of the gene causing uracil deficiency (u^-_{2614}) showed a strong increase in the first experiment, but this result did not recur in subsequent experiments. The number of back-mutations in the latter were so low that no frequencies were calculated. The observed increase in the first experiment may depend on experimental error. The slight increase of the back-mutation frequency in strain 2615 might be real according to the variation limits of the method as calculated above. In one case only did the reversion rate of the gene u^-_{2626} show some increase by EOC-treatment. Strain 2657 was unsuitable in this test for reasons discussed on page 286.

As regards the gene u^-_{2505}, the results of the experiments in Table 2 strongly indicate that the mutation frequency was increased by treatment with EOC. All 5 experiments with strain 2505 gave a clear increase of the number of back-mutations per surviving conidia. Experiment no. 5 also gave an absolute increase of the number of wild-type cells. The fact that all of these experiments gave similar results supports the conclusion that considerable experimental errors due to the method did not cause the observed increase. Such deviations should be in both

directions and should just as often cause a decrease as an increase of the back-mutation frequency. It seems much more probable that the reason was of biological origin. Because experiment no. 5 was the only one to give an absolute increase, the possibility cannot be excluded that the increase in the frequency of wild-type cells observed in the other experiments could depend on the fact that the wild-type conidia were more resistant to EOC-treatment than the uracil-less conidia. Thus, the observed increase of the frequency might depend on a selective killing. To test this possibility, reconstruction experiments were performed.

c. *Reconstruction experiments*

From the plates used in an EOC-experiment two mycelia were inoculated into liquid medium; one wild-type mycelium of spontaneous origin on the minimal plates of the control experiments, and one uracil-less mycelium from the plates for the viability test in the control. The conidia formed served as inoculum for a second set of cultures. The conidia were filtered and washed as usual, and the two types of conidia were separately suspended in distilled water and in 5×10^{-2} M EOC. The densities of the four suspensions were carefully determined in the haemacytometer. The conidia were then mixed so that the water-suspension contained equal numbers of wild-type conidia and uracil-less conidia, and the EOC-suspension the same proportions. The conidia were then handled in an identical manner as in other EOC-experiments. The number of surviving conidia of the two types was calculated after 0, 13, 24, 32, 36, 45, and 60 hours by inoculating the diluted suspensions into plates of minimal medium and minimal medium+uracil. The reresults of the reconstruction experiments with strain 2505 are shown in Figs. 2 and 3. Fig. 2 a shows that the wild-type conidia are more resistant to treatment with EOC for 36 hours (the time used in the actual experiments). However, Fig. 2 b shows that the same condition can be observed in the control experiments, where the conidia were suspended in water. It is therefore supposed that something other than the presence of EOC caused the difference of viability shown in Fig. 2 a. As the frequency of wild types was measured at the same time in the induction experiment and in the control experiment, this selective killing cannot explain the increased frequency of wild-type cells after EOC-treatment.

Reconstruction experiments with the uracil-hypoxanthine-less double mutant were also performed to determine whether any differences in

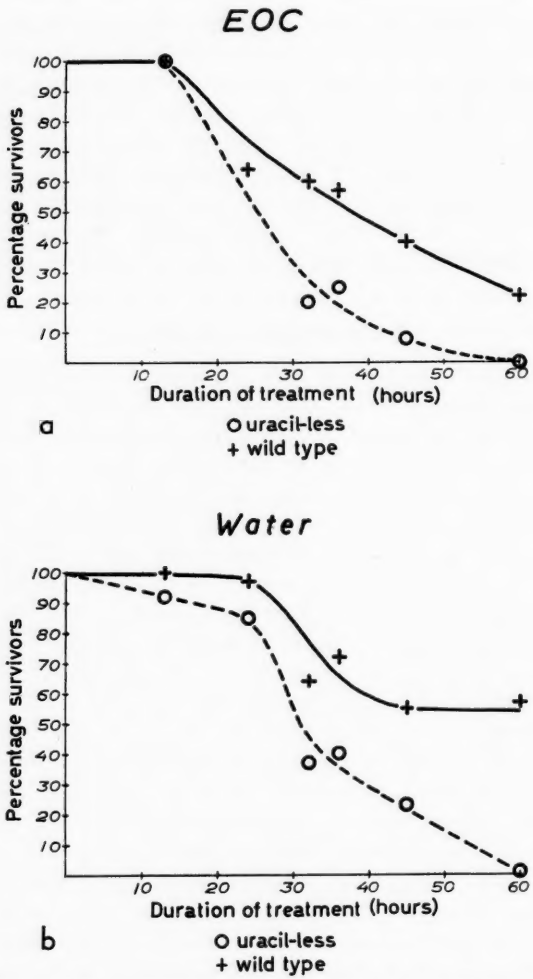


Fig. 2. Viability of the parental cells (uracil-less) and back-mutants (wild type) by treatment with a) EOC; b) water (control).

viability occurred, after treatment with EOC and with distilled water, between double mutant conidia and hypoxanthine-less conidia (reverted from $u^-_{2505}h^-_{2494}$ to $u^+_{2505}h^-_{2494}$). Fig. 3. Not even in this case could the

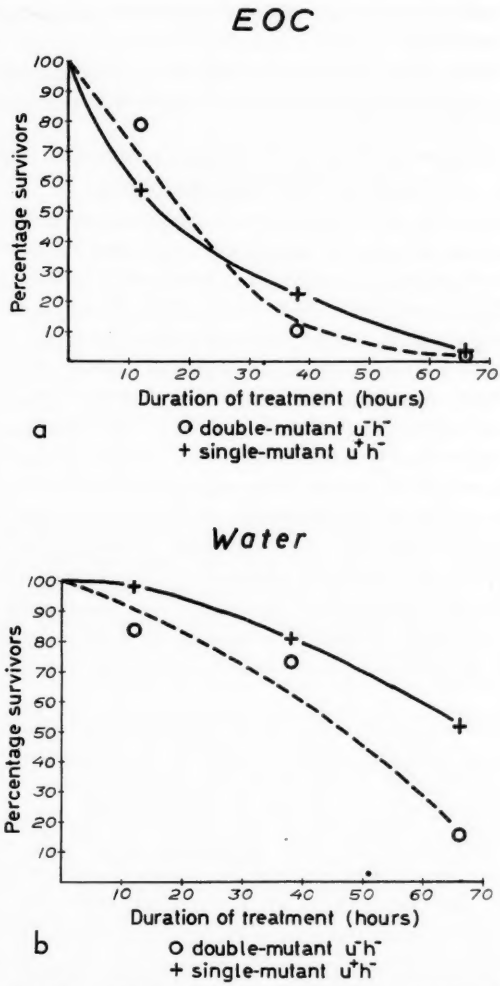


Fig. 3. Viability of the parental cells (double-mutant uracil-hypoxanthine-less) and back-mutants (hypoxanthine-less) by treatment with a) EOC; b) water (control).

differences in mortality explain the increase of the back-mutation frequency that appears after EOC-treatment. Table 2: 6, 7, 8.

The results of EOC-treatment of the uracil-methionine-less double

mutant also indicate a real increase of reversions of u^-_{2508} even in this genetic environment.

It appears from the above that evidence is available to show that EOC acts as a mutagen on certain genes under the conditions tested.

d. *Analysis of the genetic basis of spontaneous and EOC-induced back-mutations*

The back-mutation test used in these experiments gives information on the mutagenic effect of a treatment, calculated on the basis of the number of forthcoming prototrophs. However, the method does not divulge anything about the genetic basis of the physiological change.

When a biochemical mutant reverts to the wild-type condition, it is supposed to be due to back-mutation from the mutant allele to the wild-type allele. There is a possibility, however, that the restored wild-type character is caused by a suppressor mutation (WAGNER and MITCHELL, 1955), i.e., a mutation in a different locus, which means inhibition of the mutant character. If the suppressor gene and the actual mutant gene are located in different chromosomes or if they are separable by crossing over, mutant cells will also segregate in crosses between the reverted wild type and a primary wild type. In the former case the segregation will be one mutant-type ascospore per three wild-types. However, if the wild-type character is caused by a back-mutation in the mutant gene, the offspring from such a back-cross will be of only wild type.

KIHLMAN (1955 a and b) and READ and KIHLMAN (1956) stated that the chromosome-breaking action of EOC in some respects corresponded qualitatively to that of x-rays. STADLER (1941) claimed that no gene mutations could be induced by x-rays. Nevertheless, it has been shown later by DE SERRES (1958) that mutations, not distinguishable from gene mutations, have been induced by x-rays. ZETTERBERG and FRIES (1958) found a difference in the ability to revert to prototrophy between mutants of *Ophiostoma* induced with UV and x-rays. If EOC caused only chromosome aberrations, the increase in the number of wild types should not depend on real back-mutations. These latter are thought to indicate an intragenic change. However, in this case the wild-type character could instead be caused by suppressors, the formation of which is thought to be the result of chromosome aberration. If this explanation is right, crosses between primary wild type and EOC-induced wild types should give offspring that also include mutant types, whereas crosses between primary wild types and spontaneously reverted

TABLE 3. *Analysis of the offspring from crosses between primary wild type and wild types arisen spontaneously and after EOC-treatment.*

Control. (spontaneous)			After EOC-treatment. (spontaneous + EOC-induced)		
Cross no.	Type of offspring		Cross no.	Type of offspring	
	Wild type	Uracil-less		Wild type	Uracil-less
1	37	0	11	36	0
2	40	0	12	32	0
3	38	0	13	41	3
4	36	0	14	42	1
5	40	0	15	43	0
6	35	0	16	36	0
7	24	0	17	38	0
8	39	0	18	38	0
9	38	1	19	35	2
10	38	1	20	39	0

wild types should only give wild-type offspring (provided that the spontaneous back-mutations are real back-mutations, i.e. changes restricted to the gene previously damaged). To test this possibility, 10 prototrophs spontaneously arisen in strain 2505 (experiment no. 5 in Table 2), were crossed with the wild type "Andrus" [1] and the offspring were analyzed. In the same way ascospores were analyzed from crosses between the "Andrus" strain and 10 independent prototrophs obtained after EOC-treatment. (5 from exp. 2 and 5 from exp. 5 Table 2.) The result appears in Table 3. Among 10 tested *spontaneously* arisen wild types, 8 had given only prototrophic offspring by back-crosses to original wild types. From the two other crosses one auxotrophic ascospore was isolated from each. For two reasons it is unlikely that these two were mutations. 367 ascospores were isolated, and if two of these had mutated, it would mean an unusually high mutation frequency. Both are also of the same type (uracil-less). Although this is a rather frequent mutant type, the possibility that they were caused by mutations appears still more remote, when one is considering that all mutant ascospores from three back-crosses of the wild types obtained *after EOC-treatment* required just uracil. However, among the prototrophs received after treatment with EOC, those would also be included which arose spontane-

[1] "Andrus" is the sexual strain, from which the mother strain of the biochemical mutants was isolated.

ously. They ought to make up 1/6 and 1/5, respectively, of the total number of prototrophs in the two experiments.

The very weakly segregating uracil-less ascospores recall the concept of pseudoallelism (WAGNER and MITCHELL, 1955). However, the proportions obtained are of a quite different order of magnitude to those reported by PONTECORVO (1953).

It is very likely that in the crosses, where these few auxotrophic ascospores were obtained, the wild-type character of the back-mutants depends on a change in a nonallelic gene, i.e., a suppressor. However, the ratios diverge significantly from 3 : 1, and it is therefore supposed that the suppressor in question is closely linked to u^-_{2505}.

The cross experiments thus gave the following information:

1. The prototrophy owed its occurrence partly to mutation in some linked suppressor gene, and — probably — partly to real back-mutation.

2. Suppressor-dependent prototrophs were formed both spontaneously and after EOC-treatment. No evidence was presented to show a difference in the frequency of such prototrophs between the two groups.

3. If it is assumed that spontaneous reversions also are due to intragenic changes (gene mutations), there is no reason to suppose that EOC caused *only* chromosome aberrations, because the main part of the EOC-induced back-mutations could not be distinguished from gene mutations and seemed to be identical with those arisen spontaneously.

e. *Possible modes of the mutagenic action of EOC*

Considering the vigorous chromosome breaking capacity of EOC, it would be supposed *a priori* that EOC also would increase the frequency of back-mutations, if the two phenomena were caused by similar processes. However, it has been proposed by MULLER (1941) and AMBROSE and GOPAL-AYENGAR (1953) that relatively weak forces (hydrogen bonds according to AMBROSE and GOPAL-AYENGAR) combine what are supposed to constitute the genes in the chromosome, built up of those gene particles. Thus the chromosomes would be broken between these particles much more easily than within them. In the latter case a peptide bond must be broken. This requires considerably more activating energy than breaking a hydrogen bond, and is improbable also for other reasons. It is generally supposed that gene mutation means a disturbance within the "gene molecule". Our knowledge, however, of the structure of this entity is still rather fragmentary. It is certain that hydrogen bonds and other comparable forces are common in large

organic molecules and are important for the tertiary structure of proteins and the helical structure of DNA. It is possible that a structural disturbance caused by the breaking of such bonds, which are weaker than covalent bonds, may be so important for the function of the molecule that it means a change corresponding to the definition of a gene mutation.

KIHLMAN (1952) has discussed the reasons for the radiomimetic effect of purine derivatives against the background of their different abilities to break intermolecular bonds. As the same sort of bonds are important also for the intramolecular structure, the argument is for the most part applicable also to the gene-mutagenic action which these substances may possess. KIHLMAN found that the radiomimetic action of purines was connected with their ability to form molecular compounds as demonstrated by their solubilizing power. The electroaffinities of purines were investigated by PULLMAN and PULLMAN (1958), who used the results of quantum-mechanical calculations of the distribution of electronic energy levels to obtain the information. They found that purines are good electron doners and that a perfect correlation exists between their electron donor ability and solubilizing power.

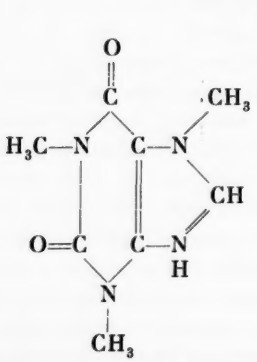
KIHLMAN (1952) also points to the possibility of an indirect effect of purine derivatives, which may disturb enzyme reactions important for the synthesis of the chromosome material. However, in later investigations (1955 a and 1956) KIHLMAN found that the chromosome breakage by purines can hardly be due to a disturbed nucleic acid synthesis. Evidence for the influence of purines on the metabolism of bacteria is presented in works by KOCH and LAMONT (1956), NOVICK (1956) and GREER (1958). KOCH and LAMONT investigated the action of a number of purine derivatives on enzymes concerned with nucleic acid metabolism in *Escherichia coli*. They found that a number of these purines inhibited the purine nucleoside phosphorylases. They are of the opinion that such inhibitory effects on the riboside phosphorylases result in a decreased concentration of the normal free purine bases in the bacteria, and that such decreases may lead to mutation by increasing the probability of the substitution of a wrong purine base in a nucleic acid. However, there was no complete correlation between mutagenic and enzyme inhibitory activity. NOVICK (1956) found that a number of purine derivatives exhibited a mutagenic action on growing bacteria, and that this action was counteracted by nucleosides and influenced by the physiological and biochemical state of the organism. GREER (1958) tested a number of substituted benzimidazoles, benzotriazoles and quinoxalines

as growth inhibitors, mutagens and antimutagens in *Escherichia coli*. Only inhibitory compounds were found to be mutagenic, except 4-nitro-6-hydroxybenzimidazole, which acted as a mutagen in noninhibitory concentrations. The mechanism of this mutagenesis was thought to include the inhibition of enzymes concerned with DNA synthesis. Mutagenesis was observed only in dividing cells. No incorporation of the supposed mutagen into DNA was found.

Each of the explanations for the mode of action of purine derivatives, discussed above, is supported by experimental evidence and there seems to be no decisive reason to exclude or prefer any of them. However, in the experiments presented here the conditions were such that an influence of EOC on the synthesis of new chromosome material must have been slight, as no growth was demonstrated during the treatment. The observed mutagenic activity of EOC may therefore favour the hypothesis of a more direct action on the gene material.

3. Experiments with caffein

a. *Methods*



1, 3, 7-trimethylxanthine (caffein)

Melting point 235°—237° C. (Handbook of Chemistry and Physics 1957.) Solubility in water at 16° C: 1.35 g/l.

According to FRIES and KIHLMAN (1948) caffein in the concentrations of 0.05—0.15 per cent causes a retarded growth of *Ophiostoma* cultures. The growth stops completely at a concentration of 0.2 per cent. In the experiments in Table 4 the concentration varied between 0.15 and 0.4 per cent. In most experiments the concentration of caffein was 0.2 per cent, i.e., the same concentration that was observed to induce forward mutations in *Ophiostoma*.

The caffein was dissolved in liquid minimal medium which also con-

TABLE 4. *Results of experiments with caffein.*

Strain	Investigated reversion	Lenght of treatment hours	Conc. of caffein per cent	Control		Treated series			
				Back-mut. per 10^6 living conidia	Total no. of back-mut.	Back-mut. per 10^6		Per cent surviving conidia	Total no. of back-mut.
						Initial conidia	Surviving conidia		
Uracil-less 2505	u^-_{2505}	2	0.4	1.0	30	1.3	1.6	69	40
	to	6	0.4			1.1	1.6	62	35
	u^+_{2505}	24	0.4			0.17	1.3	13	6
Double mutant $u^-_{2505}m^-_{2511}$		24	0.15	0.13	63	0.12	0.13	93	46
Methionine-less 2511	m^-_{2511}	24	0.2	0.18	185	0.07	0.28	26	47
	to								
Double mutant $u^-_{2505}m^-_{2511}$	m^+_{2511}	24	0.15		3			93	4
Uracil-less 2615	u^-_{2615} to u^+_{2615}	24	0.2	0.10	34	0.03	0.16	20	13
Uracil-less 2657	u^-_{2657}	24	0.2	> 100		> 100	> 100	15	
	to	24	0.2	> 100		> 100	> 100	35	
	u^+_{2657}	24	0.2	450	3600	311	311	100	2720
Uracil-less 2626	u^-_{2626}	24	0.2	650	5860	200	200	100	1594
	to	24	0.2	> 100		> 100	> 100	55	
	u^+_{2626}								
Hypoxanthine-less 2494	h^-_{2494}	2	0.2	0.28	23	0.4	0.4	100	40
	to	6	0.2			0.4	0.4	100	36
	h^+_{2494}	24	0.2			0.06	0.4	16	4
Methionine-less 2599	m^-_{2599}	2	0.2	0.52	145	0.40	0.55	72	122
	to	6	0.2			0.37	0.47	80	99
	m^+_{2599}	24	0.2			0.23	0,45	52	61
Methionine-less 2668	m^-_{2668}	24	0.2	0.10	25	0.04	0.12	35	9
	to								
	m^+_{2668}								

tained the metabolite required by the mutant strain in question, except in the opening experiments with the strains 2505, 2494 and 2511, where minimal medium only was used. Even in the experiments with the latter strains the possibility of selective growth was excluded as the high concentration of caffein prevented the conidia from growing at all. The treatment was performed in 125-ml Pyrex flasks containing 20 ml of suspension. The flasks, plugged with cotton, were placed on a shaker at 25° C. The duration of the treatment was 24 hours. In three experiments the influence of the length of the treatment was investigated.

From his experiments with caffein on *Vicia* chromosomes KIHLMAN (1951) concluded that caffein cannot penetrate the nuclear membrane, and that to achieve its radiomimetic action the cell must pass through a mitosis (when the nuclear membrane breaks up) during the treatment. In view of these circumstances it was desirable for the cells to be treated in such a way as to enable many cells to pass through mitosis, but each cell should divide only once so as to exclude the possibility of selective growth. Such a selection, however, could not be demonstrated in similar experiments by FRIES (1950). The conditions described above were reached by diminishing the concentrations of caffein and glucose until a limited growth was obtained.

b. *Results and discussion*

The results of the experiments are seen in Table 4. The information given by these experiments indicates that caffein had no mutagenic effect under the actual conditions. In the experiments with strains 2505, 2494 and 2599, where the influence of the length of the treatment was investigated, the reversion rate was not increased by prolonged treatments (cf. experiments with EOC). The slight increase of back-mutations observed in one experiment with strain 2511 and in another with strain 2615 might be real, but was not confirmed by further experiments.

The mutagenic property of caffein was first demonstrated by FRIES and KIHLMAN (1948). They found an increased frequency of biochemical mutants in *Ophiostoma*, when they treated conidia with caffein of rather high concentration, 0.2 per cent during 5 to 8 days. Caffein has also a mutagenic effect on bacteria, as shown by DEMEREC et al. (1951) and NOVICK (1956). The radiomimetic effect of caffein has been investigated by KIHLMAN (1951, 1952).

The negative results in the present experiments may depend upon treatments of too short a duration. The method used did not permit a

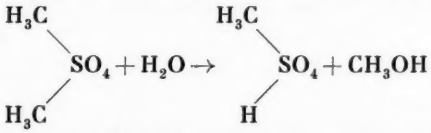
prolongation of the treatment. The growth that occurred in the control experiments, where the conidia were suspended in nutrient medium without growth-retarding substances, would have offered possibilities for an uncontrollable selection, thus diminishing the significance of the experimental results.

4. Experiments with dimethylsulfate (DMS)

a. *Methods*

Dimethylsulfate, $(CH_3)_2SO_4$, is a very reactive substance, used as an alkylating agent in organic syntheses and also as a weapon of chemical warfare. Sp. w. 1.3322, melting point −26°.8 C; boiling point 188°.3—188°.6 C; solubility in water: very slight (Handbook of Chemistry and Physics 1957).

According to KREMAN (1908) DMS is hydrolyzed by water:



A rise in temperature of 10° C increases the yield of the reaction 3.5 times. At 15° C about 90 per cent of the initial concentration of DMS remains after an hour in water; at 25° C about 75 per cent; and at 33°.5 about 30 per cent.

The mutagenic effect of DMS has been investigated by several scientists, among them KÖLMARK (1957), who worked with *Neurospora* and found a good mutagenic effect that was shown to be specific for one of the two genes investigated.

The experiments were performed at 15° C, at which temperature DMS has a good stability in water solution. The concentration of DMS was 0.002 M. Pure DMS was carefully pipetted into distilled water at 15° C. The washed conidia were suspended in 30 ml of solution in a 125-ml Pyrex flask, which was put on a shaker at 15° C. In the control experiment the conidia were suspended in distilled water. The treatment lasted 60 minutes and was considered to be stopped when the conidia were resuspended in water after centrifugation. The conidia were then washed twice in distilled water. Plates were prepared in the usual manner. The pH in the suspension was 4.7 when the treatment started and 4.4 after 60 minutes. In a water solution of DMS without conidia the pH drops to 3.5 after 60 minutes, thus demonstrating that the conidia have a buffering action.

TABLE 5. *Results of experiments with DMS.*

Exp. No.	Strain	Investigated reversion	Control		Treated series			
			Back-mut. per 10^6 living conidia	Total no. of back-mutations	Back-mut. per 10^6		Per cent surviving conidia	Total no. of back-mutations
					Initial conidia	Surviving conidia		
1	Uracil-less 2505		1.0	111	1.44	12	12	122
2		u^-_{2505} to u^+_{2505}	0.32	273	0.50	1.3	39	266
3	Double mut. $u^-_{2505}h^-_{2494}$		0.67	73	0.52	0.94	55	34
4	Double mut.		1.5	348	1.6	1.6	100	415
5	$u^-_{2505}m^-_{2511}$		0.79	435	0.82	0.95	86	394
6	Methionine-less 2511	m^-_{2511} to m^+_{2511}	0.014	10	0.33	0.61	54	212
7	Double mut.		> 100		> 100	> 100	100	
8	$u^-_{2505}m^-_{2511}$		0.03	16	0.24	0.28	86	117
9	Hypoxanthine-less 2494	h^-_{2494} to h^+_{2494}	0.09	39	0.03	0.25	13	14
10	Double mut. $u^-_{2505}h^-_{2494}$		0.47	51	0.29	0.53	55	19
11	Uracil-less 2614	u^-_{2614}		0			16	0
12		to u^+_{2614}	0.007	4	0.12	2.4	5	57
13	Uracil-less 2615	u^-_{2615} to u^+_{2615}	0.14	37	0.21	1.2	19	56
14	Uracil-less 2626	u^-_{2626}	> 100		> 100	> 100	7	
15		to u^+_{2626}	0.40	125	0.36	2.4	15	114
16	Uracil-less 2657	u^-_{2657} to u^+_{2657}	50	6700	15	70	21	1680
17	Methionine-less 2668	m^-_{2668}	0.02	4	0.44	0.60	73	52
18		to m^+_{2668}		0	1.0	2.6	40	110

b. *Results and discussion*

The results of the experiments are presented in Table 5, where the following observations and comparisons can be made:

The reversion rate of the gene u^-_{2505} was clearly increased, relative to the auxotrophy of the single mutant strain 2505. No clear increase was observed on that gene in the double mutants.

The gene m^-_{2511} showed an increase in back-mutation frequency both in the single mutant and in the double mutant. The treatment gave an absolute increase in the number of reversions. This was also observed in the experiments with m^-_{2665}.

The stability of the gene h^-_{2494} was rather weakly influenced by DMS treatment.

The back-mutation frequency of the genes u^-_{2614}, u^-_{2615} and u^-_{2626} was clearly increased after DMS treatment.

On strain 2657 only observations on the decrease in viability could be made. See p. 286.

It is evident from the above that the mutagenic effect of DMS varied considerably for different strains and genes. This also agrees with KÖLMARK's (1957) observations on two genes in *Neurospora*. He also discussed the mechanism of the mutagenic effect and the reasons for its gene-specific action. ZAMENHOF (1957) showed that DMS inactivated the transforming principle by alkylating primary amino groups of the nitrogenous bases and by esterifying the phosphate groups. It is probable that the mutagenic effect of DMS on *Ophiostoma* is caused by similar disturbances in the gene material.

5. Experiments with ultraviolet irradiation

a. *Methods*

The conidia were taken from cultures in the logarithmic phase, washed twice in sterile distilled water and resuspended in the same liquid to give a density of 5×10^7 conidia/ml. 13.3 ml of the suspension were pipetted into a sterile petri dish with a diameter of 85 mm. For the irradiation a 15 W Luma germicidal lamp (low pressure mercury vapour lamp emitting 90 per cent of its light at 2537 Å) was used. As no exact data of its energy output were available, its biological effect was compared with a Philips TUV 15 W lamp. On this basis it was calculated that the dose given in the experiments in Table 6 was about 250 000 erg/cm^2. The irradiation was made in a dark room at room temperature. The conidia were kept in constant motion to give a uni-

TABLE 6. *Results of irradiation with ultraviolet light.*

Exp. No.	Strain	Investigated reversion	Control		Treated series			
			Back-mut. per 10^6 living conidia	Total no. of back-mutations	Back-mut. per 10^6		Per cent surviving conidia	Total no. of back-mutations
					Initial conidia	Surviving conidia		
1	Uracil-less 2505		4500	4000	2700	6700	40	1199
2			1.4	172	53	206	16	5000
3	Double mut. $u^-_{2505}h^-_{2494}$	u^-_{2505} to u^+_{2505}	0.65	75	11.2	32	35	1310
4	Double mut.		0.5	93	200	200	100	1521
5	$u^-_{2505}m^-_{2511}$		1.5	348	11.5	28	41	2000
6			0.62	166	14.6	54	27	4000
7	Methionine-less 2511	m^-_{2511}		0	6.2	14	44	2000
8		to	0.03	19	3.2	5.8	56	1780
9	Double mut.		> 100		> 100	> 100	41	
10	$u^-_{2505}m^-_{2511}$	m^+_{2511}	0.096	26	2.5	9.4	27	688
11	Hypoxanthine-less		0.28	23	1.1	2.4	47	94
12	2494	h^-_{2494}	230	1070	101	390	26	435
13		to	0.1	7	3.1	10.9	28	156
14			0.11	11	1.95	3.0	65	194
15	Double mut. $u^-_{2505}h^-_{2494}$	h^+_{2494}	0.06	7	1.9	5.4	35	133
16	Uracil-less 2614	u^-_{2614} to u^+_{2614}	0.51	122	6.5	13.6	48	1566
17	Uracil-less 2615	u^-_{2615}		0	6.8	19	36	1240
18		to u^+_{2615}	0.14	37	8.5	21	40	2305
19	Uracil-less 2626	u^-_{2626}	0.60	134	0.80	1.3	62	183
20		to u^+_{2626}	0.40	125	1.9	2.7	72	607
21	Uracil-less 2657	u^-_{2657}	4.7	746	4.3	5.6	77	682
22		to u^+_{2657}	50	6700	30	60	50	4000
23	Methionine-less 2668	m^-_{2668} to m^+_{2668}	0.08	12	1.6	9.0	18	248

form exposure. After irradiation the suspension was shielded against bright white light so that no photoreactivation could occur. The control was handled in an identical manner except for the UV-irradiation.

b. *Results and discussion*

The experiments made it evident that irradiation with ultraviolet light increased the back-mutation frequency of all investigated genes. (Except u^-_{2657}. See p. 286.) This increase was usually very strong, the only exception being u^-_{2626}, which gene in two experiments has been shown to be fairly resistant to UV-irradiation. Ultraviolet light is a well-known mutagen for microorganisms, and in the experiments here, it was of interest to compare its effect with that of the other tested mutagens. The result showed that UV-irradiation was generally the most mutagenic of the treatments tested.

An interesting problem, not investigated here, is whether or not the sensitivity for UV-irradiation might be positively correlated with the fact that the biochemical deficiencies of all the mutant strains used have been induced by UV-irradiation. A positive correlation would mean that the cells by the first UV-irradiation were specifically disposed for UV-induced reversion of the characteristic mutant character.

Further investigations of the effect of irradiation with ultraviolet light are in progress.

6. General conclusions

In the experiments with the three mutagenic treatments, EOC, DMS and UV-light a gene-specific effect was observed, i.e., one mutagen greatly increases the back-mutation frequency of a particular gene, but only to a limited extent when acting on another gene. Certain genes that were most affected by UV-irradiation did not show the greatest sensitivity by DMS-treatment, e.g., h^-_{2404}; u^-_{2618}. Many of the very UV-sensitive genes were not affected at all by EOC-treatment. (Cf. KÖLMARK, 1953; GLOVER, 1956; HARTMAN, 1956.)

GLOVER (1956) observed in experiments with *Escherichia coli* that the genetic background influenced the stability of a gene. He supposed that this indicated an indirect action of mutagens in general. Some of the experiments described here support his results. The gene m^-_{2511} was not clearly influenced by treatment with EOC when it was a part of the genome of strain 2511 (Table 2). When it was included in the uracil-methionine-less double mutant its back-mutation frequency was further

increased. A reverse situation exists for the treatment of the gene u^-_{2505} by DMS. As a single mutant it is clearly influenced whereas it is protected in some way against the mutagenic effect of DMS when it is included in the double mutants $u^-_{2505}h^-_{2494}$ and $u^-_{2505}m^-_{2511}$ (Table 5). The differences observed can be understood, if we make the following supposition: Mutation is the final result of biochemical reactions, abnormal for the cell, which have been started or influenced by the mutagenic treatment. In these reactions components take part whose existence and concentration are dependent on the genetic constitution. Thus it can be imagined that the disposition for mutation can also be influenced in the same manner as e.g., morphological characters when the genome is modified.

This way of regarding the matter is also attractive when the mutagenic effect of EOC is compared with that of DMS. These two substances are widely different in their chemical activities. EOC is chemically rather indifferent whereas DMS is extremely reactive. However, the fact that this difference in chemical activity is not always positively correlated with mutagenic activity was demonstrated in the experiments with the gene u^-_{2505} (Tables 2 and 5). The objection can perhaps be raised that the treatments with EOC and DMS are not comparable, since the concentrations (on a molar basis) and the length of treatment were different. However, the lethal effects of the two treatments were of the same strength. In the author's opinion it is probable that the metabolic damage is of comparable extent, when such a state is reached that the continuation of the life-processes becomes impossible. In such cases it should be reasonable to compare the specific mutagenic effect of the two substances.

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SUMMARY

The ascomycete *Ophiostoma multiannulatum* has a habit of growth that makes it suitable for a back-mutation test. Such a test is described, and a statistical analysis is presented. The influence of a high background of reverted cells is discussed.

Experiments have been performed with 9 biochemical mutant strains, in which spontaneous reversions were observed, and 2 double mutants obtained by mating experiments. The influence on the back-mutation frequency of treatments with 8-ethoxy-caffein (EOC), caffein, dimethylsulfate (DMS) and irradiation with ultraviolet light was investigated. These experiments gave the following results: 1. The greatest increase in back-mutation frequency of the investigated genes was in general obtained by irradiation with ultraviolet light. 2. DMS was usually a much weaker mutagen than UV-light. 3. EOC had in comparison with UV-irradiation a slight mutagenic effect on certain genes whereas other genes did not increase their spontaneous back-mutation frequency. Reconstruction experiments excluded the possibility of a selective killing. Mating experiments indicated that the main part of the mutations induced by EOC-treatment are real back-mutations. However, wild-type cells caused by mutation of a suppressor gene occur both spontaneously and after EOC-treatment. The mode of action of EOC is discussed. 4. By treatment with caffein no clear increase in the spontaneous back-mutation frequency was observed.

The gene-specific effect of the mutagens was confirmed. The observation of GLOVER (1956) that the genetic background is of importance for the stability of genes in *Escherichia coli* was shown to be also valid for *Ophiostoma*. The supposition that mutagens can have an indirect mode of action is accepted and is applied to a comparison between the mutagenic capabilities of EOC and DMS.

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CYTOLOGICAL AND EXPERIMENTAL STUDIES IN KOELERIA

I. KOELERIA PUBESCENS

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THE genus *Koeleria*, comprising about 60 species, has been comparatively neglected by cytologists. Very few chromosome numbers are known, and a more detailed cytological analysis has never been performed on any of the species of *Koeleria*. BÖCHER (1943) in a short note gives the chromosome numbers of some strains of *K. glauca* and *K. pyramidata*, grown at the experimental fields belonging to the University of Copenhagen. These experiments have been continued, and during the last five years a great number of species belonging to this genus have been experimentally studied. The present paper deals with some results concerning the species *K. pubescens* (LAM.) P. BEAUV. This highly polymorphous annual species seems very well suited for cytological studies as it was found to comprise races containing B-chromosomes. Its distribution is restricted to the Mediterranean area, in the western part of which it is particularly common. Several infraspecific taxa have been described; the present strain belongs to the typical variety.

MITOSIS

The plant used for these cytological studies originated from natural populations in France (Languedoc, sent through the Botanical Gardens, Montpellier). After germination of the seeds, the seedlings were planted in separate pots, one plant in each, and given the characters a—p. Each plant was fixed in order to see the frequency of cytological deviations which might occur. At the end of the growing season all the surviving plants were dried for further taxonomical studies.

For chromosome counts, root-tips were fixed in chromosome-acetic-formalin (MÜNTZING's modification of NAVASHIN's Fluid), embedded in paraffin and sectioned at 15 μ. The slides were stained according to the Feulgen method after hydrolysis for 30 minutes.

The chromosome number not previously established was found to be 2n=12 (Fig. 1). This is the first time that the basic number 6 has been found in the genus *Koeleria*, which, like most other genera belonging to the tribe *Aveneae*, has the basic number 7. The discovery of this new number will be discussed below.

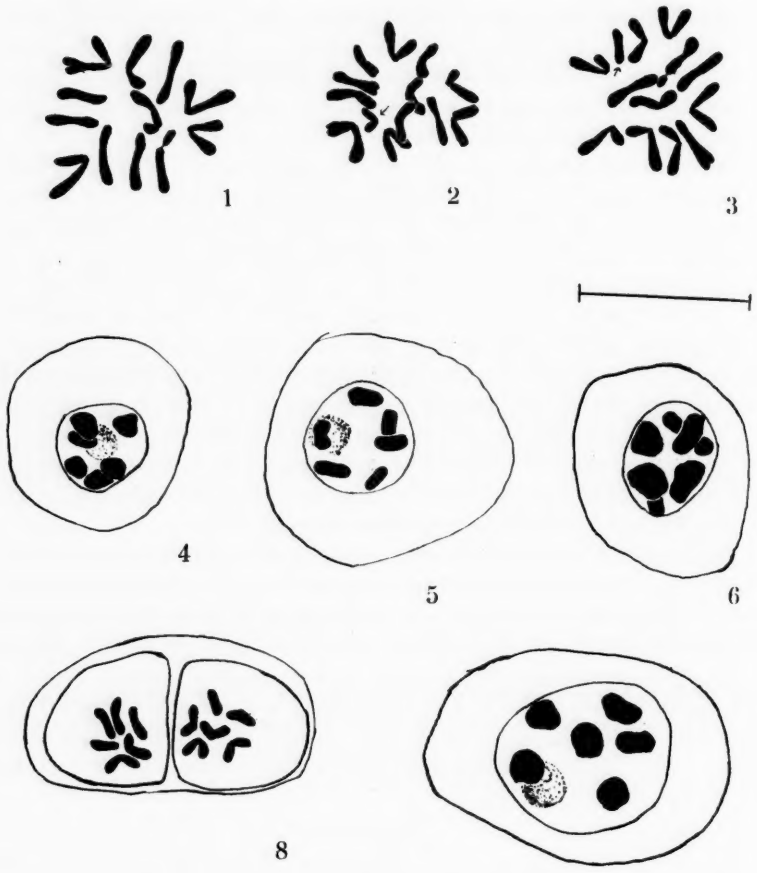
The chromosome size was the same as that commonly found within the *Aveneae*, and the chromosomes had all median or submedian kinetochores. Frequently one of the chromosomes showed a comparatively large satellite. Perhaps two of the chromosomes had this shape, but it could not be ascertained in any of the metaphase plates studied, and in the majority of the cases no secondary constriction could be observed.

From the present strain of 15 plants, 12 were found to have the chromosome numbers 2n=12 reported above, whereas 3 plants showed 2n=12+1B. In all, 404 mitotic metaphase plates were counted from 50 root-tips. The results are summarized in Table 1. The figures in brackets refer to the numbers of metaphase plates counted in each root-tip. Thus, of the plant b, 28 metaphase plates were counted from 4 root-tips, 5, 11, 10 and 2 metaphases plates in each.

The B-chromosome is only slightly smaller than the chromosomes belonging to the normal complement. In Figs. 2—3 it is indicated with an arrow. Its shape and relative size corresponds to those of the standard B-chromosome of rye (MÜNTZING, 1944; LIMA-DE-FARIA, 1948) and of

TABLE 1. *The occurrence of B-chromosomes in the strain examined.*

Plant	No. of root tips	No. of Metaphase plates	Chromosome No.
a	3	17 (3, 7, 7)	12+1 B
f	3	20 (4, 9, 7)	12+1 B
p	3	30 (6, 15, 9)	12+1 B
b	4	28 (5, 11, 10, 2)	12
c	7	37 (4, 5, 14, 4, 4, 1, 5)	12
d	2	4 (1, 3)	12
e	6	48 (9, 11, 10, 7, 3, 8)	12
g	5	39 (8, 11, 8, 2, 10)	12
h	8	45 (5, 4, 12, 4, 9, 6, 3, 2)	12
i	2	15 (5, 10)	12
k	1	11	12
l	5	21 (3, 6, 2, 8, 2)	12
m	4	47 (16, 13, 10, 8)	12
n	4	29 (10, 12, 5, 2)	12
o	2	13 (3, 10)	12



Figs. 1—3. Root-tip mitoses. — Fig. 1, plant b, (2n=12); Figs. 2—3, plant a, (2n=12); one B chromosome indicated by an arrow. — Figs. 4—8. Meiosis in pollen mother cells. — Figs. 4—7, late diakineses, nucleolus and nuclear membrane visible; Fig. 8, second metaphase. The scale is 10 μ.

Poa alpina (MÜNTZING and NYGREN, 1955). In Fig. 2 it is seen that the kinetochore is median in the B-chromosome.

In all the root-tips studied from one plant the same number of B-chromosomes were found, viz., one. Thus there is a clear difference between *K. pubescens* and, e.g., *Centaurea scabiosa* studied by FRÖST

(1956), who found a slight variation within the plant of the B-chromosomes.

In *Plantago serraria*, BÖCHER, LARSEN and RAHN (1955) found a type of B-chromosome very much like that in *K. pubescens*. In *Plantago serraria*, however, it deviated from the chromosomes of the normal complement in being present in the resting nuclei as an overstained body. This has never been observed in *Koeleria pubescens*.

MEIOSIS

Only one of the plants was fixed for the study of meiosis, viz., plant c. This showed a regular meiosis with 6 bivalents as appears in Figs. 4—7. Unfortunately, none of the three plants with accessories were fixed. The author, however, intends to grow more strains of *K. pubescens* in order to study the behaviour of the B-chromosomes in meiosis and to explore the question whether more than one of these accessories may be found in other strains.

EXPERIMENTAL CULTIVATIONS

In the cultivation experiments it was not possible to show any significant differences between the plants with B-chromosomes and those without. From the field notes it can be mentioned that all the plants started flowering 2 ½ months after the seeds had germinated. In the rainy autumn of 1957 nearly all the plants started a second flowering, as appears from Fig. 9. The plants which died first were: i, h, and p, i.e., two without and one with B-chromosomes, whereas the plant that lived longest was a, which had B-chromosomes.

In an experiment with the same strain in 1956, in which no chromosome counts were made, one of the plants was transferred to the laboratory. It was left there during the winter and flowered again the following spring, after which it died. It was often observed that Mediterranean annuals are able to hibernate in a house with a moist, cool environment.

DISCUSSION AND CONCLUSIONS

A. *Cytotaxonomy and phylogeny.* The chromosome number 2n=12, found in *Koeleria pubescens*, deserves further attention, as it is remarkably rare not only in the tribe *Aveneae* but also in the whole family of *Gramineae*. The basic number 6 is found in the small tribes *Oryzeae*,

Fig. 9. *Koeleria pubescens.* Dried specimen from the experimental field. 1/4 size.

Centotheceae, *Arundineae*, and *Bambuseae*. Within *Aveneae*, n=6 has been found with certainty only in *Schismus calycinus* and in 26 species of *Danthonia*. To these two isolated cases we can now add the present species.

The genus *Koeleria* was divided by DOMIN (1907) into two subgenera, viz., *Airochloa* and *Lophochloa*. All species hitherto studied cytologically belong to *Airochloa*, while *K. pubescens* together with a dozen other annual Mediterranean species belongs to *Lophochloa*. Some other of these annuals have been studied by the author (unpubl.) but they also belong to the common 7-series. DOMIN is of the opinion that no close relationship exists between the two subgenera of *Koeleria*, in any case not more so than between the other representatives of the *Koeleriinae*,

viz., the genera *Schismus* and *Eatonia*. It is interesting to note that the deviating basic number n=6 is also found in the related genus *Schismus*, a genus of small Mediterranean annuals, but it is too early at present with our scanty cytological knowledge to give any support to DOMIN's theory.

DOMIN furthermore emphasizes that the annual *Lophochloa* group is by no means phylogenetically younger than the perennial *Airochloa*. The author is not of the same opinion. All the characters of *Lophochloa* seem to be a further development of the ancestral *Koeleriinae* showing reduction in nearly every part in some of the species. In *K. pubescens* this reduction has also affected the chromosome number. It is a common evolutionary trend in several genera to develop from perennials to annuals, and at the same time to show a reduction in the basic chromosome number. As examples mention may be made of *Crepis* (BABCOCK, 1947) and *Coronilla* (LARSEN, 1955, 1956); furthermore, a review on this subject has recently been published by SHARMA, A. K. & A. (1959).

B. *The B-chromosomes*. The study of the B-chromosomes is at present attracting the attention of cytologists. Every day new reports appear of species with these accessories. The family of Gramineae, which on account of its many economically important species, has been fairly thoroughly investigated; it shown a remarkably high degree of species having B-chromosomes.

The B-chromosomes of *Koeleria pubescens* are interesting in that they have nearly the same size as the chromosomes belonging to the normal mitotic complement. BOSEMARK (1957) studied the B-chromosomes of different species of grasses. It seems that those of *K. pubescens* are more or less similar to those of *Alopecurus pratensis* in BOSEMARK's study, except for the fact that no positive heteropycnosis was observed.

Koeleria pubescens is not the first species of *Koeleria* to show B-chromosomes. TATEOKA (1954) found 2n=14+1B and 2n=14+2B in *K. gracilis*. The author has studied several strains of this widespread species from several parts of Europe (unpubl.), and was able to confirm the results of the Japanese botanist. It is a very interesting fact that in some species of *Koeleria* B-chromosomes are present in a high frequency whereas in other species, e.g., *K. glauca*, they have never been met with. This is the more remarkable as *K. glauca* is the most thoroughly studied species. The present author, after having counted material from all over *Koeleria*'s western distribution area, has arrived at the same result as BOSEMARK (1957), viz., there is in *Koeleria glauca* a complete lack of strains with B-chromosomes.

SUMMARY

(1) The chromosome number of *Koeleria pubescens* (LAM.) P. BEAUV. is found to be 2n=12.

(2) In three out of 15 plants belonging to the same population one B-chromosome was found.

(3) The B-chromosome is about 3/4 of the length of the normal chromosomes and is constantly found in all the root-tip cells studied in the three plants in question. No positive heteropycnosis was found.

(4) Meiosis in a plant without B-chromosomes was normal: meiosis involving B-chromosomes was not studied.

(5) No morphological or biological differences could be proved to occur between plants with and without B-chromosomes.

(6) Evolutionary trends within the genus *Koeleria* are briefly discussed.

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